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Review article

Diversity in common epithelial carcinoma, simplicity in sarcoma and leukemia

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Abstract

In some type of leukemia and sarcoma, cancer development mechanism is straightforward; aberrant activation of single oncogene transforms cells. Such oncogenes are master genes which function upstream of transcriptional cascade. On the other hand, cancer genome analyses clearly show that that of common epithelial cancers such as lung adenocarcinoma is related to accumulations of mutations in "non-master" oncogenes, leading to heterogeneity in aggressiveness and clinical behavior among their patients. Common epithelial carcinomas do not arise from mutations in specific oncogenes but from accelerated growth rate as a result of summation of growth advantage of each driver mutations on a different composition of oncogenes. Because most of human cancers are of epithelial origin, this heterogeneity gives us a challenge both in understanding the molecular mechanism of carcinogenesis and in the treatment of carcinoma.

Key words: heterogeneity, common epithelial carcinoma, chromosomal translocation, oncogene

Introduction

Although 85% of human cancer is derived from epithelial cells, which cover the surfaces of skin, gut, and other tube structures throughout our bodies, most information about cancer and its mechanisms were not derived from studying cancers of these cells, but rather from less common cancers such as leukemia and sarcoma, owing to the relatively simple mechanisms of leukemogenesis and sarcomagenesis. Leukemia and some types of sarcoma are associated with the reciprocal chromosomal translocation. Many oncogenes have been cloned from the breakpoints of these chromosomal translocations. These oncogenes are "master genes" that regulate transcription, signal transduction, and cell growth; many of them normally mediate developmental processes. Whereas activation of oncogenes, inactivation of tumor suppressor genes, and formation of new fusion proteins are relatively straightforward tumorigenic mechanisms in leukemia and sarcoma, those of common epithelial cancers are caused by accumulations of many types of mutations in "non-master" oncogenes, leading to a wide diversity of carcinogenic mechanisms, which makes them more difficult to identify and clarify.

Burkitt lymphoma

Burkitt lymphoma is a B-cell malignancy characterized by very aggressive clinical behavior and a unique histology, which make its diagnosis relatively easy. It was classified as a special type of high-grade non-Hodgkin lymphoma. The molecular characteristics of Burkitt lymphoma are associated with specific reciprocal chromosomal translocations, the most common variant of which is t(8; 14)(q24; q32), which accounts for approximately 85% of cases and affects genes for c-myc and immunoglobulin heavy chain. This chromosomal translocation aberrantly activates the *c-MYC* oncogene through the immunoglobulin heavy chain enhancer. All Burkitt lymphomas are characterized by disregulated *c-MYC* genes. However, *v-MYC* was initially found in a retrovirus that causes avian myelocytomatosis; *c-MYC*, the homologous human sequence, was then identified in the specific chromosomal translocation breakpoint of Burkitt lymphoma. *c-MYC* is a tran-

scription factor that regulates about 15% of all human genes. The role of c-MYC as a general transcription factor was highlighted when it was identified as one of the four gene products (Yamanaka factors) that, in combination, could reprogram somatic cells into pluripotent stem cells (Takahashi et al. 2006). Aberrant *c-MYC* activation results in co-activation of downstream genes and changes the fate of once-differentiated cells (B cells in case of Burkitt lymphoma), eventually to permit clonal growth of lymphoma cells. Here, the *c-MYC* oncogene is an example of a master gene, which functions upstream in a transcriptional cascade and controls expression of many downstream genes, aberrant expression of which can completely change a cell's fate. Its activation presents the master gene model of cancer development (Rabbitts 2001). In this model, one oncogene mutation can lead to a specific type of cancer.

T-cell acute lymphoblastic leukemia

Another example of the master gene model of cancer development is the specific chromosomal translocation associated with T-cell acute lymphoblastic leukemia. In this case, the oncogene *LMO2* is activated by a reciprocal chromosomal translocation under the influence of a T-cell receptor α- or a δ-chain enhancer. This T cell-specific reactivation of master transcriptional regulator *LMO2* completely changes the T-cell's fate. In normal development, *LMO2* mediates hematopoietic stem cell development from hemogenic endothelial cells in the embryonic dorsal aorta. The LMO2 protein is part of a transcription factor complex that activates many downstream regulators of hematopoiesis and angiogenesis, including c-kit, VE-cadherin, and angiopoietin. LMO2 expression can trigger blood cell development, and thus functions as a master transcriptional regulator of hematopoiesis and angiogenesis. Aberrant LMO2 expression in specific T cells endows them with a hematopoietic stem cell-like feature: the potential of self-replication, through reactivation of downstream genes (McCormack et al. 2010, Cleveland et al. 2013). The phenomenon of resetting through aberrant LMO2 activation was recently highlighted in the induction of hematopoietic stem cells (iHSCs) by six genes transfected into committed lymphoid and myeloid progenitors (Riddle et al. 2014). Thus, a single growth-increasing mutation in the *LMO2* oncogene in T cells results in neoplastic transformation of T cells, and explains the very aggressive behavior of T-cell acute lymphoblastic leukemia. T-cell development requires somatic DNA recombination in T-cell receptor genes. If these recombination errors involve master genes, such as *LMO2*, the resulting phenotype could be serious. Several oncogenes other than *LMO2* have been identified from breakpoints of specific reciprocal chromosomal translocations in leukemia and sarcoma and most of them were identified as mediators of the transcription factor hierarchy or of the developmental process. In all cases, one mutation in these master genes could completely change the cell's fate and give it enormous growth advantage. As the accumulation of oncogenic mutations is less likely in blood cells and mesenchymal cells than epithelial cells, more growth-enabling mutations on the master genes are needed for cancer development in blood and mesenchymal cells. Unsurprisingly, successful identifications of oncogenes were achieved through studies of leukemia and sarcoma. Burkitt lymphoma is an aggressive form of B-cell malignancy and T-cell acute lymphoblastic leukemia is the T-cell counterpart of aggressive leukemia. These two malignancies typify the master gene model of cancer development.

Myxoid liposarcoma

Specific chromosomal translocations affect not only hematological malignancies but also some sarcoma types. One such sarcoma is myxoid liposarcoma with a specific chromosomal translocation at t(12; 16) (q13; p11). Among four major histologically categorized liposarcomas (well differentiated, myxoid, round cell, and pleomorphic), myxoid liposarcoma is characterized by its unique vascular architecture. Its specific chromosomal translocation and the resulting FUS-CHOP fusion protein primarily instigates sarcoma development. FUS-CHOP works within tumors as a transcriptional activator. This is another mutated master gene model of cancer development.

Heterogeneity in common epithelial carcinoma

Somatic mutations in so-called oncogenes are involved in the development of common epithelial carcinomas. However, in this context, oncogenes are defined as genes which mutations offer cells a growth advantage. Some mutations, for example those that truncate C-terminals of the protein products, inactivate the coded protein. In these cases, the genes are often called tumor suppressor genes. However, we do not distinguish tumor

suppressor genes from oncogenes here. Mutations that confer these growth advantages are called driver mutations. Driver mutations are not necessarily in the coding region (exome) of the oncogenes; frequently, they are found in the promoters and other regulatory sequences, and can thus modify the oncogenes' expression levels and patterns, and therefore give growth advantages. To date, a variety of oncogenes have been identified though many approaches. They affect cell growth, cell cycling, signal transduction, transcription, DNA replication, cell death, and apoptosis. Although physiological functions of oncogenes vary, they commonly affect the cell growth rate. Driver mutations may activate or inactivate protein product expression; overall, they allow cells to grow faster than they normally would. In this article, oncogenes are subclassified into master genes and non-master oncogenes. As mentioned before, master genes usually encode transcription factors which work on the upstream of transcriptional cascade and effect the expression of a number of downstream genes. When they are aberrantly expressed or activated, they are able to completely change the cell fate and eventually give cells enormous growth advantage. They normally play a critical role in developmental process and decide the cell fate. Only one hit on master genes has a potential to transform cells. *MYC*, *LMO2*, *TAL1*, *ABL* and *RUNX1* are among master genes. On the other hand, non-master oncogenes confer cells relatively smaller amount of growth advantage when they are mutated and activated compared. They usually comprise growth factor receptors (such as epidermal growth factor receptor and vascular endothelial growth factor receptor) or a member of signal transduction pathway. Epithelial cells have several distinctive features: their turnover is much faster than for other cell types; they regenerate from common stem cells and swiftly differentiate into various functioning cell types; and they divide much faster than other cell types. These characteristics of epithelial cells enable or force them to accumulate somatic mutations within their DNA. As epithelia cover most body structure surfaces, they can be exposed to various environmental carcinogens (mutagens), such as ultraviolet light and cigarette smoke. Thus, the simple master-gene model of cancer development does not necessarily apply to carcinogenesis (development of common epithelial cancers), as the single mutated oncogene (master gene) that leads to a specific cancer need not occur here. Alternatively, acumulation of somatic mutations on "non-master" oncogenes in different composition is more likely to be the cause of cancer development from epithelium. Of course, the involvement of master gene mutation in carcinogenesis is not excluded. It is less likely simply because master genes comprise only small portion of all oncogenes. Therefore, determining the genetic basis of each case of carcinogenesis requires comprehensive analysis of each cancer genome, which has been enabled by recent technological progress. Actually, major international cancer genome projects to identify a set of oncogenes involved and new oncogenes (if any) are currently underway (Lawrence et al. 2013). These analyses found no common development pathway to specific epithelial carcinoma types; rather, they usually found involvement by many non-master oncogenes and identified several genes that mutated at significantly high frequencies (Vogelstein et al. 2013). In the case of adult, esophageal squamous cell carcinoma, about 75 non-synonymous mutations (mutations that would be expected to alter gene products) per tumor were found. Although determining which mutations are driver mutations for these cancers is difficult and most of the mutations are not driver mutations, some mutations were in the better-known oncogenes, such as *TP53* or *KRAS*. The presence of strong mutagens drastically increases the average number of mutations per tumor. Even when each driver mutation's contribution to the cell's growth advantage is small, their accumulation will turn normal cells into carcinoma cells eventually. This pattern may confound the chief aim of the cancer genome analyses, i. e., discovery of new oncogenes. Although oncogenes that harbor small driver mutations might be identified, their individual contributions to the cell's growth advantage could not be as great as those of master gene mutations.

Between "reactive" and "neoplastic"

Current concepts of carcinogenesis clearly distinguish between reactive processes and neoplastic disease. Hepatocellular carcinoma may develop from infection by hepatitis B or C virus, and be preceded by chronic hepatitis from virus infection and resulting liver cirrhosis. As hepatocytes are repeatedly regenerated in association with the chronic inflammation, they accumulate somatic mutations on oncogenes more frequently than do normal liver cells; when accumulation of driver mutations reaches a certain level, the cells undergo a neoplastic change. In this scenario, the

reactive process is continuous with the neoplastic process. The cancer genome atlas shows about 40 non-synonymous mutations per hepatocellular carcinoma. Although identifying the final mutation that transformed the reactive process to the neoplastic one is unlikely, accumulation of small contributions to cell growth ultimately leads to the liver malignancy. Carcinogenic processes are closely connected to recurrent regeneration triggered by chronic inflammation. The near future may bring genome analysis of chronic inflammatory diseases that lead to carcinogenesis, such as liver cirrhosis, metaplastic gastritis (*Helicobacter pylori* infection to gastric adenocarcinoma), and chronic gingivitis (oral cavity inflammation to squamous cell carcinoma). The distinction between reactive process and neoplastic disease may be less clear in the genome context, just as molecular distinctions between benign and malignant tumors became less clear in studies of stepwise colorectal carcinogenesis.

Grade, aggressiveness, and cancer genomics

Cancers caused by the aberrant expression of master genes usually behave uniformly, depending on the clinical entity. For example, the clinical course and behavior of Burkitt lymphoma is uniform among its patients. Chronic myeloid leukemia, another master gene disease based on the *BCR-ABL* mutation, showed an almost identical phenotype in its chronic phase. The reason for their uniformity lies in the influence of the master gene mutation on the etiology of diseases (which, in this context, includes aberrant expression by chromosomal translocation). This feature allows these cancers to be similarly treated. In contrast, clinical manifestations of gastric carcinoma vary among patients. Some very well differentiated adenocarcinomas of the stomach were once classified as gastric adenomas and behave almost as benign tumors; partial mucosal resections can completely cure such cases. Much more aggressive forms of gastric carcinoma invade the stomach wall deeply and metastasize very quickly. Thus, gastric adenocarcinoma encompasses a variety of tumors with a wide range of malignancy and aggressiveness. Structural information (structural atypism) based on histological findings is integrated in subclassification of stomach adenocarcinoma, then further classified into well, moderately, or poorly differentiated adenocarcinoma. Although poorly differentiated adenocarcinomas are generally more aggressive than well differentiated ones, this tendency depends on the case. In other forms of carcinoma, such as endometrial adenocarcinoma, a sub-classification grading system is applied to endometrioid adenocarcinoma. If the proportion of the adenocarcinoma that shows solid growth (i.e., has little tendency to form glands) exceeds 50%, the carcinoma is considered to be grade 3, and is generally more aggressive than its grade 1 counterpart, which would be better differentiated (i.e., capable of forming glands). Cellular atypism (enlargement of nucleus, uniformity of nuclei size within tumor, high N/C ratio, prominent mitosis counts, and nucleoli) is of higher value in subclassifying transitional cell carcinoma of the urinary bladder. Grade 3 transitional cell carcinoma shows marked cellular atypism and is generally more malignant than grade 1 tumors. All these sub-classifications should be considered in their treatment because of the wide clinical variety of common epithelial carcinomas. Genome analysis of common epithelial carcinomas gives a rationale for this clinical variety. Gastric cancers have a median of 53 non-synonymous mutations per tumor; endometrial cancers have 49. The cancer phenotype is the result of accumulation of relatively small driver mutations on non-master oncogenes; it represents the summation of the growth advantage conferred on the tumor by each driver mutation. The amount of growth advantage of each driver mutation varies, depending on which gene was mutated and the mutation type and site. If the mutation is within the activating sites of the master genes, its impact on the cell's growth advantage is enormous, although as driver mutations on non-master oncogenes accumulate to a certain level, the degree of growth advantage becomes strong enough to transform cells. The features of epithelial cells, as discussed before, permit such accumulation. Because the simple master gene model of cancer development does not apply to most carcinogenesis, their aggressiveness varies according to the total growth advantage from all the mutations. When the growth advantage that each driver mutation contributes can be calculated and handled quantitatively, a cancer genome atlas of each patient can be used as the ultimate grading system for cancer aggressiveness. Each carcinoma has its own intrinsic malignancy.

Common epithelial carcinomas do not arise from mutations in specific oncogenes

The chronic phase of chronic myeloid leukemia

(CML) reflects the master gene *BCR-ABL*, and is another example of the master gene model of cancer development. BCR-ABL fusion protein formation presents the common initiation pathway for cancer development in the CML chronic phase. The advent of the tyrosine kinase inhibitor, imatinib, gave us the most optimistic view for its treatment. However, the constitutive activation of tyrosine kinase in blood cells sometimes itself leads to genomic instability and the accumulation of secondary mutations on oncogenes, resulting in progression to advanced-stage CML, known as the accelerated stage and the blast stage. At this advanced stage, CML no longer shares the common pathway activated in blood cells. The cancer development mechanism of most common epithelial carcinomas is similar to that of the advanced-stage CML, which presents a challenge in cancer therapeutics because one cannot expect to stop cancer cell growth by blocking the common pathway of cell growth in one type of carcinoma. The genomic profile of one cancer varies from another and the oncogenes involved in carcinogenesis differs case by case, even in the same type of carcinoma. We must sometimes consider more than several pathways of cell growth advantage in one cancer.

How many oncogenes are left to be discovered by cancer genome study?

The first list of oncogenes came from studies of oncogenic viruses. Retroviruses, which have the potential to transform cells in vivo and in vitro, have the virus oncogenic sequences (*v-onc*), later those homologous sequences were discovered in human cells and are called cellular oncogenes (*c-onc*). In this article, an oncogene is simply defined as a gene in which a mutation gives its cell a growth advantage—a definition that includes tumor suppressor genes and master genes. In cancer genome studies, the new definition of oncogene should include genes that mutate significantly more often than would be expected at random in cancers. This definition for oncogenes would pick up genes that initially appear irrelevant to cell growth, such as those that encode olfactory receptors and the muscle protein titin (Lawrence et al. 2013). A deeper study of the gene products could reveal unexpected roles for these proteins, which may lead to their reclassification into the classical list of oncogenes (as defined in this article). Alternatively, another explanation of their higher mutation frequency in cancer might be found. In recent studies of lung carcinoma, nearly all genes found to mutate at significant frequency have already been discovered to be oncogenes (Vogelstein et al. 2013). However, even if a new master type oncogene is not discovered in cancer genome studies, functional studies of products of frequently mutated genes and identifying oncogenes (as alternately defined) is invaluable. A quantitative approach for carcinogenesis by measuring the cell growth advantage from each driver mutation is essential, considering the mutational heterogeneity in common epithelial carcinoma.

Synergic growth advantage, high score driver mutation

According to classic epidemiologic studies, development of ordinary solid tumors requires at least five to eight driver mutations. As discussed above, determining which mutation in a cancer genome is the driver mutation is difficult. If a non-synonymous mutation appears in a well-documented site of an already identified oncogene, it can reasonably be supposed to be the driver mutation. In other cases, determining if each mutation is a driver mutation is currently beyond our powers. The pediatric tumor medulloblastoma requires only eight non-synonymous mutations per tumor, as a median, among which, one to two mutations are considered to be driver mutations. Because only the exome of this cancer genome was sequenced, some driver mutations of medulloblastoma could have been missed. More critically, these "one to two" driver mutations could be high score mutations in cancer development, in which their contribution per mutation to cell growth advantage is much greater than the average contribution—conceivably almost equivalent to master gene mutations. The importance of analysis of low non-synonymous mutation tumors (such as acute myeloid leukemia, chronic lymphocytic leukemia, glioblastoma, neuroblastoma, medulloblastoma, and rhabdoid tumor) cannot be overstated. However, the possibility of a synergic effect on cell growth by two or three mutations should not be ignored. Historically, the transfection of *MYC* combined with *RAS* transformed cultured fibroblasts in vitro.

Cancer therapeutics in the cancer genome era

In conventional cancer chemotherapy, the common features of cancer cells, such as faster cell division, DNA synthesis, and replication of normal cells, are targets of drugs, such as inhibi-

tors of nucleic acid metabolism and DNA synthesis, or blockers of cell division. Conventional drugs are effective for liquid tumors such as leukemia, and solid tumors of germ cell origin. However, on other types of cancer, especially common epithelial carcinomas, the effect is very limited. For master gene cancer such as chronic myeloid leukemia, specific inhibitors for master gene product have successfully opened a new stage for cancer therapeutics. However, considering the heterogeneity of common epithelial carcinomas, we cannot reasonably expect a master gene-specific approach for their therapies. In carcinoma, different sets of drugs may be needed to treat the same type of carcinoma (for example, esophageal squamous cell carcinoma). Tailor-made chemotherapy for common epithelial carcinomas that depend on the type of driver mutations could be the therapeutic choice in the cancer genome era.

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