Editorial

Evolutionary acquired robustness and vulnerability in cancer genome: negligible negative selection in carcinogenesis

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Cancer is a disease involving the accumulation of somatic genetic alterations accompanied by clonal evolution (1,2). A wide range of oncogenes and tumor suppressor genes are known to be mutated, deleted, rearranged, and/or amplified in most cancers. These genes have been identified by functional analyses (transformation of NIH3T3), genetic linkage analyses, and structural genomic analyses such as cytogenetics. Functional characterizations of these mutated genes have revealed that the mutations contributing to carcinogenesis (so-called driver mutations) confer malignant phenotypes on tumor cells, including unlimited and anchorage-independent cell growth, metastasis, lack of apoptosis, and de-differentiation (3).

Around the end of the last century, several polymerase chain reaction (PCR)-based modalities were developed to survey the somatic genetic alterations in identified oncogenes and tumor suppressor genes [for an example, see (4)]. Cancer scientists had been frustrated by the inability to identify driver mutations in many of their cancer samples, especially pediatric ones, although they did their best to screen the somatic genetic alterations in candidate genes. The most frequently mutated gene in cancer genomes, *TP53*, shows somatic mutations in 25–60% of cancer cases [for a review, see (5)].

In 2007, the first comprehensive study of genome-wide mutations in breast and colon cancers was reported (6). In that study, a total of 20,857 transcripts from 22 breast or colorectal cancer samples were sequenced. To achieve this, 125,624 PCR primers had to be synthesized for 6,196 transcripts and Sanger sequencing had to be performed on all of the amplicons. This work made the discovery that not many driver mutations can be found in cancers (around 15 mutations per tumor) and most of the mutated genes are unique to individual patients. The authors referred to the mutation spectra of the 22 cases as a landscape and characterized only a handful of frequently mutated genes as "mountains" and several dozen infrequently mutated genes as "hills" in cancer tissues.

One major paradigm shift in this field was the identification of defective mismatch repair (MMR) in hereditary nonpolyposis colon cancer (HNPCC) syndrome (7,8). Cancer-specific repeat unit insertions or deletions in repetitive sequences are frequently found at the microsatellites or short tandem repeat sequences in the DNA of the HNPCC tumor genome. The genes conferring susceptibility to HNPCC are related to the MMR pathway, which repairs misincorporated nucleotides during the S phase of DNA replication. Although dysfunction of this pathway would be expected to result in huge increases of somatic point mutations in HNPCC tumor genomes, genome-wide increases of point mutations in HNPCC tumors were not revealed by conventional mutation searches, targeted to known cancer driver genes. Recently, the Cancer Genome Atlas Network elucidated that colon cancers with defects in MMR show a much higher mutation rate than MMR-normal tumors (9).

Based on the idea that genomic instability is the major cause of carcinogenesis, we have a lot of questions. Are there patients with malignant tumors lacking any driver mutations? Do the differences of mutation signatures depend on the origin or cause of tumors? Is there any mutual exclusiveness between the driver mutations among

cancer cases? How many druggable driver mutations are there in a cancer? How many mutations can be borne by a cancer? Does negative selection occur in cancer? This final question is critical because it was convincingly argued that the mutator mutation phenotype caused by biallelic inactivation of the MMR genes might not contribute to the initiation of carcinogenesis (10). Sieber *et al.* deduced that hypermutation caused by defective MMR would increase deleterious mutations in cancer cells and it would not be advantageous in carcinogenesis. The argument that defective MMR may not contribute to the initiation of carcinogenesis is based on the following premises: (I) the carcinogenic process basically involves monoclonal expansion with the accumulation of driver mutations in a clone; (II) the carcinogenic process resembles Darwinian evolution (natural selection) that would be constituted by both positive and negative selections; and (III) the increase in the mutation ratio upon defective MMR may increase deleterious mutations in cancer cells sufficiently to delay the process of carcinogenesis by inhibiting cell physiological processes. In addition, the genome instability itself may play a negative role in cell proliferation. For example, DNA mismatches caused by defective MMR activate cellular guardian systems, including apoptosis, growth arrest, cell cycle checkpoint, and cellular senescence; these activated systems would inhibit the ability of tumor cells with DNA damage to proliferate. However, recent studies have revealed that MMR deficiency causes the hypermutation phenotype by a failure to repair DNA replication errors and is involved in the tolerance for cell growth arrest induced by DNA base lesions caused by some anticancer drugs, such as S_N1 DNA alkylators and cisplatin, through impairment of the function of checkpoint kinase [for a review, see (11)]. Therefore, it has been critical to resolve the issue of whether there is substantial negative selection in carcinogenesis to estimate the significance of MMR genes in this process.

The advent of next-generation sequencing (NGS) technologies has introduced a new epoch in this field of study; theoretically, most point mutations, chromosomal rearrangements, and small insertions/deletions in cancer cells can now be detected by whole-genome sequencing. Moreover, the phylogenetic comparison of the numbers of reads between mutated sequences and wild-type ones would provide further information: clonal expansion and diversification of the carriers of the driver mutations (12). Many of the questions can be readily answered using the accumulated data of somatic genetic alterations in cancer tissues generated by NGSs. Alexandrov *et al.* reported the identification of 21 mutation signatures (now numbering more than 30) in the catalog of somatic mutations in cancer genomes (13). Some of these signatures are strongly related to carcinogens or carcinogenic metabolic pathways, such as tobacco smoking or APOBEC activation, and many of them are also restricted to certain types of tumor (13). It has also been reported that some malignant tumors do not have any driver mutations. For example, pediatric tumors such as medulloblastoma frequently show a lack of obvious driver mutations (14). Mutual exclusion of the mutated genes in tumor cells has also been observed among genes that have the same cellular functions or pathways. Efforts to detect the mutual exclusiveness of driver mutations using bioinformatic approaches are actively underway (15).

Martincorena *et al.* focused on the issue of whether substantial negative selection occurs in carcinogenesis (16). Deleterious mutations in the essential genes can be endangered for the survival of tumor cells and be good targets for cancer chemotherapy. Because of the analogy between carcinogenesis and Darwinian evolution, statistical tools in evolutionary biology have been applied to analyze the large amounts of data on somatic genetic mutations in cancer tissues. Martincorena *et al.* applied the dN/dS ratio to identify any sign of negative selection among mutations in whole-genome or whole-exome sequencing identified in Tumor Cancer Genome Atlas (TCGA) data of 29 types of cancers from 7,664 patients, after the fine filtering of the data. According to this paper, 97–98% of the nonsynonymous mutations found in cancers are neutral and up to 0.5% of the mutations would be lost during carcinogenesis. These lost mutations seem to be subjected to negative selection. Therefore, Martincorena *et al.* concluded that negative selection is largely absent for coding regions in the cancer genome (16).

Why is negative selection against somatic single-nucleotide alterations so rare in carcinogenesis? Here, we propose one possible explanation: negative selection may mainly affect the dominant negative mutations in genes essential for cell survival.

We define "dominant negative" as when a mutated gene product inhibits the corresponding wild-type gene product or its downstream effector molecules and causes the loss or significant decrease of functions of the gene product in the cell. This may not involve a simple loss-of-function mutation; instead, the mutated product could override the wild-type product, which might be rather rare. For example, a premature stop codon in an open reading frame may be useful to generate such dominant negative proteins

that have lost active domains but retained other functions intact. However, nonsense-mediated decay would inhibit the production of truncated proteins, so that potential dominant negative products may not appear in the cell (17).

As described by Sieber *et al.* and Martincorena *et al.* (10,16), multiploidy or copy number gains of chromosomes in cancer cells are frequently observed, which may overcome the simple loss of function of the genes caused by point mutations. These "simple loss-of-function mutations" in tumor suppressor genes should be enumerated as "driver mutations" by the dN/dS method reported previously (16). Martincorena *et al.* indicated that only haploid essential genes characterized elsewhere (18) showed significant signs of negative selection. Blomen *et al.* revealed that only 1,734 genes are essential in the mammalian genome, as determined by the random insertion of transposons in two human haploid cell lines (18). Moreover, a study by Wang *et al.* also indicated that a similar number of genes, 1,878, are essential (19). Wang *et al.* also showed that most of these essential genes in a haploid cell line do not exhibit haploinsufficiency in diploid cells (19). In other words, most of the loss-of-function mutations in one allele of essential autosomal genes can be supplemented by the remaining wild-type allele and be tolerated by the host cell. In such a case, only the dominant negative mutations that can inhibit the remaining wild-type copy of the corresponding gene can decrease fitness and be subjected to negative selection. Therefore, cancer cells exhibiting genomic instability that increases somatic point mutations, such as defective MMR, would need to have stable ploidy to maintain the remaining wild-type alleles of essential genes that may suffer from loss-of-function mutations (but not dominant negative ones) in the other alleles. Indeed, it is well known that the defective MMR and chromosomal instability that causes allelic imbalances are mutually exclusive in most cancer tissues (10), indicating that diploidy should be essential for the survival of cancer cells with defective MMR. Second, the reason why there is negligible negative selection on single-nucleotide alterations in carcinogenesis is that the sites that could potentially confer vulnerability, regarding single-cell survival, have already been substantially reduced from the human genome during the long history of natural selection. For example, gene duplications generate the homologs or paralogs in the genome and many of which are not essential because of functional redundancy (18,19).

What impact has the study by Martincorena *et al.* had on clinical oncology? The negligible negative selection in carcinogenesis and the fact that only half of cases have driver mutations in several hundred known cancer-contributing genes might be bad news for drug developers and patients. Martincorena *et al.* indicated that, in terms of the presence of driver mutations in the nearly 400 known genes, they were found in only half of analyzed cases. Considering the high cost and long period required identifying drugs that target a molecule specifically and efficiently, it would be prohibitively difficult to develop molecular-targeting anti-cancer agents for all of the minor driver genes that may be found in a patient's cancer. However, as Wood *et al.* suggested around 10 years ago, the use of drugs targeting a pathway rather than driver mutations would be an efficient way to develop new anti-cancer therapies (6). Hypermutation may place a genetic burden on the essential genes in a cancer cell. Because negative selection on single-nucleotide alterations is negligible in cancer cells, deleterious mutations in essential genes should also accumulate in the surviving cancer cells. Such half-damaged genes and their associated pathways can be targets for cancer chemotherapy of individuals. Actually, the colorectal cancers with defective MMR show better prognosis than that with intact MMR (20), implying the potential vulnerability of the cancer cells with defective MMR by the accumulation of deleterious mutation. So-called "synthetic lethal" combinations of cellular pathways can be identified in some cases (21). Genome-wide mutation searches of somatic genetic alterations in cancer should provide essential information for designing personalized combination chemotherapy for individual patients. In conclusion, big somatic mutation data of cancers with functional annotations have provided important insights into carcinogenesis, evolutionary biology, and therapeutic strategies. Some analytical tools have already been developed to identify the synthetic lethality in the cancer genome and to design personalized therapeutics based on the large amount of data available on the mutations in cancer samples [for example, (22)]. Further accumulation of the cancer mutation data will contribute better detection of synthetic lethality and consequently, the improvement of cancer patients' chemotherapies.

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