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Perspectives

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Present and future of cancer biomarkers

Abstract: The cancer biomarker field appears to be stagnant. Very few, if any, new cancer biomarkers have been introduced into clinical practice the last 20 years. The reason is that most of the newly discovered cancer biomarkers are inferior in terms of sensitivity and specificity to the classical cancer biomarkers that we currently use. The revolutionary technologies of proteomics, genomics, and other omics did not deliver on the promise to discover new and improved cancer biomarkers. However, more recently, the explosive growth of whole genome and exome sequencing has provided for the first time nearly complete mutational landscapes of many cancer types, in thousands of samples. We now know that many of these mutations are only found in cancer. It is thus possible that the mutant proteins encoded by these genes may represent the long-sought, highly specific cancer molecules that we may envision to use as cancer biomarkers. I here speculate that modern mass spectrometry may have the necessary sensitivity and specificity to detect mutant proteins in various biological fluids for the purpose of diagnosis, prognosis, and disease monitoring.

Keywords: cancer biomarkers; mass spectrometry; mutant proteins; *p53* gene mutations; whole genome sequencing.

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Introduction

The interest of cancer societies, granting agencies and diagnostic companies to discover, validate and clinically apply cancer biomarkers is still very high. This is based on the premise that cancer, if diagnosed and treated early can be cured, or at least, transformed to a chronic disease. However, the expectation that new cancer biomarkers could be quickly discovered and introduced into clinical practice by using the powerful omics technologies of the last 20 years has not been fulfilled. This is due to the fact that most of the newly discovered cancer biomarkers do not meet the required sensitivity and specificity specifications that are necessary for clinical implementation. Consequently, the vast majority of these biomarkers are either abandoned or are not clinically validated.

Many researchers have expressed pessimistic views regarding discovery and clinical application of novel cancer biomarkers. Indeed, the number of newly approved cancer biomarkers by the Food and Drug Administration (FDA) is very limited. In this Editorial, I examine the possibility of combining the revolutionary new technologies of whole genome and whole exome sequencing with the highly powerful technology of mass spectrometry to identify mutant proteins in proximal fluids or in the circulation. It appears that these mutant proteins represent truly specific cancer biomarkers, since they should not be present in the normal state. I further mention in this review the technical difficulties of this approach and possible ways of overcoming them. In the end, only time will show if this option is a new and novel viable way for cancer diagnostics.

Current status of cancer biomarkers

A handful of cancer biomarkers are currently used in the clinic. Examples include prostate-specific antigen for prostate cancer, carcinoembronic antigen for gastrointestinal, breast and lung cancer, CA125 for ovarian cancer, CA19.9 for pancreatic cancer and CA15.3 for breast cancer. Unfortunately, none of these markers is either sensitive or specific enough for population screening or early

diagnosis. Most, if not all, of these markers are recommended in practice guidelines for patient management, such as evaluation of a patients' response to a specific therapy or earlier detection of relapse in patients who have already been treated [1]. So, there are still many clinical unmet needs with cancer biomarkers.

The promise of "omic" technologies to discover new and better biomarkers

Ten to 15 years ago, it was thought that the revolutionary technologies of microarrays, genomic sequencing, proteomics, epigenomics and metabolomics will facilitate the discovery of new and improved biomarkers [2]. Unfortunately, no new major cancer biomarker has been approved by the FDA over the last 20 years. I believe that part of the reason is that the task of identifying new and highly sensitive and specific biomarkers has been underestimated. In order for a biomarker to perform well in clinical practice, it has to fulfill certain criteria, such as excellent sensitivity (i.e., identification of most patients with the disease), excellent specificity (i.e., test negativity in non-diseased population) and other characteristics, such as high positive or negative predictive value. The latter are dependent not only on sensitivity and specificity, but also on disease prevalence. For example, an ovarian cancer biomarker that is suitable for screening asymptomatic individuals should have a specificity >99%, in order to avoid too many false positives, due to the rarity of the disease in the general screened population (<1:1000). In addition, the test should have at least 80% sensitivity, in order to detect most of the cancers. Such characteristics for a tumor marker are very difficult to achieve, since at least for the markers that we know today, none of them is absolutely specific for cancer, and many of these markers are elevated in some benign conditions. In addition, we have been rushing to discover novel biomarkers by using the new technologies, sometimes forgetting other principles of good laboratory practices, such as use of the appropriate samples, appropriate statistics, etc. As I mentioned in my previous commentaries, many of the newly discovered biomarkers reported in the literature (and some of them in very prominent journals) have been subsequently found to be associated with either pre-analytical, analytical, post-analytical or bioinformatic artifacts [3, 4]. These discoveries have since been declared as "false discovery" which means that the original results could not be reproduced in subsequent studies. I have documented many such examples of "false discovery" in my previous publications [3, 4]. Additionally, even if a discovery is true, it may still not be suitable for clinical use because the characteristics of the biomarker, such as sensitivity and specificity, are not good enough for clinical practice. One example to illustrate the point is that we cannot tell a patient based on his/her clinical characteristics the likelihood of relapse of their cancer after primary therapy is 20% but the use of a biomarker may increase their chance to 25% or so. These are called "non-actionable" predictions and have no clinical value since the clinician will not change their decision making based on small changes of such probabilities.

Whole genome sequencing technologies for discovering new biomarkers

The new, high-throughput sequencing technologies have allowed us for the first time to examine the genome wide mutational spectrum of many cancers. We have now whole exome or whole genome sequencing data for over 5000 cancers, of at least 25 types [5, 6]. The numbers are increasing by the day. This very rich information allows cross-comparisons of mutations within a cancer type and between cancer types to identify the most frequently mutated genes in cancer. For example, there are approximately 120 genes that are frequently (>2% frequency) mutated in various types of cancer and each cancer type is characterized by approximately six to 20 frequent mutations. It has also been shown recently that these mutations carry prognostic information [6, 7]. For example, some are correlating with poor prognosis while others are associated with better prognosis. So, to conclude, these new whole genome sequencing technologies are enriching our knowledge on genes that are mutated and likely involved in the pathogenesis of cancer. We hope that this information will ultimately also lead to the discovery of novel biomarkers, in addition to discovering novel therapeutics that target pathways to which this mutated genes are participating.

The role of proteomics for cancer biomarker discovery and validation

Proteomics is a wonderful technology which allows delineation of very complex proteomes in a matter of days. It is now a very simple task to identify 3000–4000 proteins in a proteome of cell lines, tissues, biological fluids, etc.

It is also possible to compare proteomes between non-diseased and diseased populations to identify differences that may be used to discover and validate novel biomarkers [2]. One of the main limitations of proteomics is that it is mostly a qualitative technique and the existing quantitative proteomic approaches are not as yet as precise or accurate as our best methods for identifying and quantifying proteins, such as ELISAs. Another major limitation of proteomics is that although you can monitor specific proteins in complex biological mixtures, in actual practice, the sensitivity of mass spectrometry in measuring a single protein in a very complex mixture, such as serum, is still lagging behind the best ELISA methodologies by two to three orders of magnitude. Since most of the biomarkers that we are using today are present in ng/mL concentrations, and we anticipate that new and improved cancer biomarkers may be present at much lower concentrations in serum and other bodily fluids, it is next to impossible to quantify proteins with mass spectrometry in complex mixtures if their concentration is below, let us say, 100 ng/mL, without previous sample fractionation and/or enrichment. This is a major bottleneck, which I hope will be overcome in the future through new instrumentation and improved sample preparation techniques.

The concept of "rare" tumor markers

Up until recently, many research groups, including my own, were trying to identify a single biomarker for a single cancer, with the hope that its sensitivity (ability to identify patients with disease) is very high so that no patients are missed. However, we are now starting to realize that this is probably a utopia, since we are learning that cancer, even for a single organ, is not a homogenous disease but rather, a very heterogeneous mix of various histotypes [8, 9]. For example, the most common form of ovarian cancer, epithelial ovarian cancer, comes in four different histological types, high-grade serous (the most common), endometrioid, clear cell and mucinous, which are characterized by very different biological and genetic backgrounds. We are now thus dealing with at least four different diseases rather than one, and it is highly unlikely that these four different diseases will all be captured with a single biomarker. Even within a certain histotype, there are differences in various tumors in terms of genetic variability, mutational spectrum, etc. I have recently shown with some experimental data that there are tumor markers which are elevated in a very small proportion of patients (e.g., 2%–5%). I have thus postulated that these markers, which were thrown in the garbage basket in the past for low sensitivity (even if they had very high specificity) could be used for personalized monitoring in those patients for which the markers are informative [10]. In the future, by using whole genome and other technologies we may be able to explain as to why these markers are only elevated in a few patients, but until then, the cataloging of these rare cancer markers may be in order. I envision that these rare cancer markers could be measured early when patients are diagnosed to see which ones are the most informative and then use them for personalized monitoring of responses to therapies.

How genomics and proteomics could be combined in the quest to find new and improved cancer biomarkers?

As mentioned earlier, genomics has provided us with the unprecedented capability to identify numerous mutations in cancer genes, some of them more frequent than others. As also mentioned earlier, none of the current biomarkers are elevated only in cancer and are absent from normal cells. However, mutant genes are encoding for mutant proteins and these mutant proteins may be "true tumor markers", since they should be present only in cancer; not in the normal state. Although this needs to be further proven, small studies have shown that this is the case, i.e., mutations of cancer genes are not found in normal tissues [11]. Technologically, it is possible to identify mutant proteins through their tryptic proteolytic fragmentation in vitro and selected reaction monitoring mass spectrometry. It may now be possible to identify mutated peptides in the circulation, tissues or bodily fluids, which are associated with malignancy, with an expected 100% specificity. Of course, we anticipate that the sensitivity of such methods will likely be very low since the mutation that one can monitor with mass spectrometry may be a rare one (i.e., found in only 1 out of 30 or 50 cancers). However, mass spectrometry also has this wonderful capability of being a multiparametric method and it allows monitoring of hundreds of (mutant) peptides simultaneously [12]. We may envision a cancer panel that may be powerful for diagnosis and prognosis. I am optimistic that the combination of the rich information of genomics with the protein/peptide detection capability of mass spectrometry may yield new technologies that are addressing the unmet clinical needs in the cancer biomarker field.

Closing remarks

Although people are getting a bit frustrated with our inability to discover successful new biomarkers, we need to keep in mind we are still in the early stages of exploring the omics technologies. It may well be that we need to mature more in thinking and technologies before tackling successfully this problem. I am optimistic that great cancer biomarkers are still hiding somewhere. Hopefully, with the aid of new technologies and ingenuity we will be able to find them, validate them, and then introduce them into clinical practice.

Another consideration to keep in mind is the time needed from discovery of a biomarker to implementing it in clinical practice. It took more than 10 years for CA125, CA19.9 and PSA [13] and more than 15 years for PCA3 prostate cancer test and a new multitarget stool DNA screening test for colorectal cancer [14, 15]. Rittenhouse et al. [14] and Pavlou et al. [16] provide timelines and milestones for introducing new cancer biomarkers to the clinic.

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