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WHITE PAPER

Proteomics and the development of precision medicines against cancer

Cancer is a heterogenous mixture of diseases characterized by, among other things, the abnormal, uncontrolled growth of cells derived from otherwise healthy tissues (Hanahan 2022). Although cancer cells sometimes grow into balls of cells and stop there (so-called benign tumors), often they gain the ability to disperse throughout the body, seed the growth of other tumors, disrupt the function of a variety of organs, and ultimately kill patients. In this white paper, we discuss how proteomics can drive the creation and use of “precision medicines” that target the processes enabling cancer growth and stop cancer in its tracks.

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Introduction

Cancer is a heterogenous mixture of diseases characterized by, among other things, the abnormal, uncontrolled growth of cells derived from otherwise healthy tissues (Hanahan 2022). Although cancer cells sometimes grow into balls of cells and stop there (so-called benign tumors), often they gain the ability to disperse throughout the body, seed the growth of other tumors, disrupt the function of a variety of organs, and ultimately kill patients (Figure 1).

Cancer cells generally acquire the initial ability to multiply beyond their normal boundaries through mutations in genes that control cell growth. Such initial mutations set cells on a path to expand and divide rapidly, gain more mutations, attract blood vessels, and travel to other tissues (Hanahan and Weinberg 2011).

Recognizing the origin of cancer in such "driver" mutations, scientists have made many efforts to develop cancer treatments that target the proteins encoded in these mutated genes. Unfortunately, these efforts have only been successful for cancers with very strong driver mutations. For instance, a fusion of 2 genes can cause chronic myeloid leukemia (CML). The encoded fusion protein continuously signals for immune stem cells to grow and divide thereby leading to leukemia (Rumpold and Webersinke 2011). By targeting this protein with chemical ("small molecule") drugs, so-called "precision medicines," cancer growth is disrupted and most patients go into remission for years (Hochhaus et al 2017).

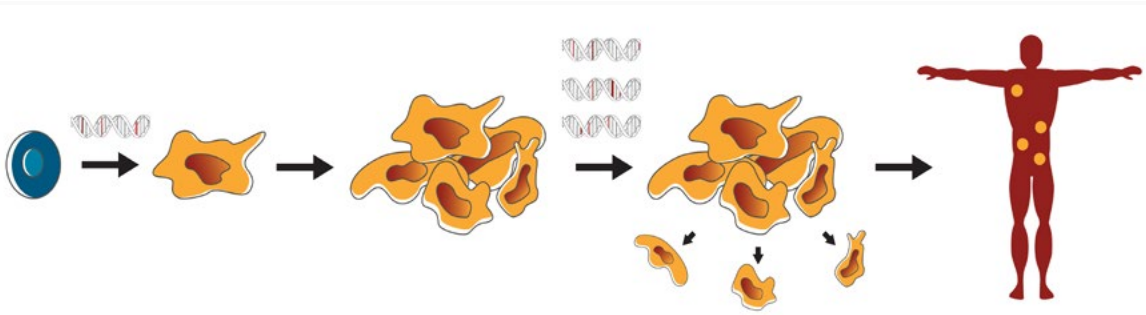


Figure 1. Driver mutations and cancer progression.

Driver mutations (red) cause healthy cells (blue) to transform into cancer cells (yellow) that grow and multiply beyond their normal, healthy boundaries. Although a single mutation (red) may be responsible for cancer development, there will likely be many other mutations (pink) that are inconsequential and it is hard to tell the difference between such mutations using genomic sequencing data alone. As cancer progresses, cancer cells acquire more mutations that enable them to continue to grow and spread throughout the body. Once again, the mutations that drive this process (red) come with inconsequential mutations (pink).

Deciphering the means through which this fusion protein causes CML was by no means easy and it took decades to develop drugs against it. Nonetheless, many cancer-associated mutations cause disease in much less straightforward ways and are harder to develop precision medicines against. For example, cancer cells often have mutations comprising large inversions, duplications, deletions, insertions, or even translocations of long stretches of the 23 human chromosomes. One such translocation causes the gene fusion described above, but these chromosomal abnormalities can impact many genes at once and it is rarely obvious which of encoded proteins should be targeted with a drug.

Other times, there are many small changes to genes throughout a cancer cell's genome. Many of these mutations will not have any impact on the encoded proteins and looking at DNA sequences alone is only the starting point for a scientist trying to understand their functional consequences.

Even if a scientist can identify a driver mutation in a specific gene, the encoded protein might not be easy to target with a therapeutic. For instance, scientists may not have chemical compounds that can bind to and affect the function of the mutated protein in any significant way. Thus, they may wish to look at the downstream impacts of this mutation to find proteins that are altered indirectly. These may be easier to target. For example, if a driver mutation in a protein known as a transcription factor causes it to enhance the production of growth-promoting proteins, scientists might want to target these growth-promoting proteins instead of the transcription factor. In this case, knowing the driver mutation alone would be insufficient

for scientists to know what growth-promoting proteins to target. They would need to know how the production of other proteins changed as a result of the driver mutation. Proteins with increased abundance in cells containing the driver mutations might make good drug targets.

Overall, sequencing the DNA of cancer cells can give scientists clues as to the cause of a cancer and, in rare cases, may give them a precise protein to target therapeutically. However, in many cases, knowing information only about the static genome is neither enough to diagnose the particular type of cancer nor guide the best treatments.

Proteomics can complement DNA sequencing (genomics) and give researchers much more information about the mix of proteins found in cancer cells. Indeed, a cell's "proteome" is its precise protein make-up including protein abundances, protein modifications, and protein distribution throughout the cell. Advanced proteomics technologies, like those being developed at Nautilus, aim to rapidly catalog the proteomes of cells grown in the lab or derived from patients. Below, we discuss how advanced proteomics technologies can have far reaching impacts on cancer and can improve therapeutic outcomes.

Proteomics and cancer diagnostics

When a patient presents with an abnormal, possibly cancerous growth, doctors may begin their investigation into whether or not it's dangerous by taking a biopsy, a sample of the growth. Often, they'll analyze the sample under a microscope and look for physical features associated with uncontrolled growth. Increasingly, they'll also sequence the DNA from the biopsy and thereby identify any mutations known to be associated with certain types of cancer.

This information may be enough to tell physicians whether or not the growth is dangerous, but only rarely do these efforts provide certainty about the precise molecular cause of the growth. In the rare cases where the molecular cause is obvious from DNA sequencing, doctors can prescribe their patients with treatments that directly target the proteins involved. This can be highly beneficial for patients and, in fact, studies have shown that such "precision medicines" are more effective than treatments chosen without the aid of sequencing (Morash et al 2018).

Currently this is only possible for a small subset of cancer patients because mutations in DNA do not directly tell scientists how the encoded proteins are affected. A mutation is generally necessary for an effect but not all mutations are driver mutations. Using advanced proteomics technologies, physicians can get information that directly shows how proteins are perturbed in cancer cells. While traditional proteomics technologies don't always have the sensitivity, accessibility, or throughput required to analyze proteins from small patient samples and large numbers of patients, up-and-coming technologies have much higher sensitivity and aim to catalog the vast majority (95% in Nautilus' case) of the human proteome in as little as 24 hours.

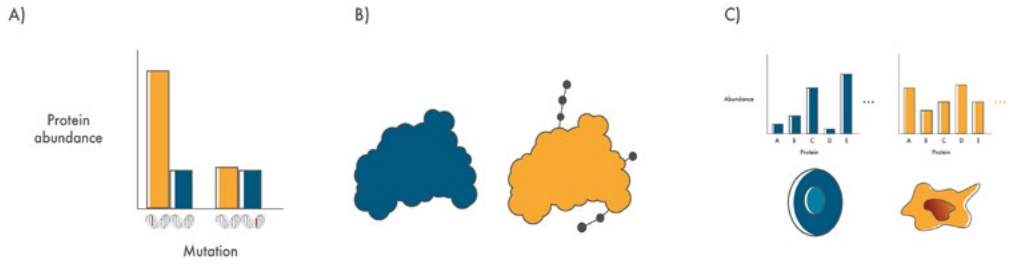


Figure 2. Questions that proteomics can help answer.

A) Is the protein encoded in a mutated gene produced in cancer cells in a significant amount? B) Does a mutation result in changes to post-translational modifications on proteins? C) Does a mutation result in wide-scale proteome changes in cancer cells?

This will give researchers a variety of actionable pieces of information including:

- Whether proteins encoded in mutated genes are produced in any significant amount. Cancer cells often contain mutations in multiple genes and it is far from obvious which of these might be mechanistically involved in the cancer. Using proteomics, researchers can check if any of these proteins are actually produced and assess their production relative to matched healthy cells. Those that are expressed at higher levels may be good drug targets while those expressed at lower levels might give insights into the molecular pathways involved in that particular cancer.

- Knowledge of proteome-wide post-translational modifications. Although there are roughly 20,000 protein-coding genes in the human genome, the proteins encoded in these genes can be modified in many ways once they are "translated" or produced from this information. These post-translational modifications (PTMs) have many functional impacts on proteins. They can make proteins more active, change where they're located in the cell, and even mark them for destruction. With their many roles, PTMs have far-reaching impacts on cancer.

  In particular, a PTM known as "phosphorylation" is often involved in activating signaling pathways that cause cells to grow. By using proteomics to discover what proteins are phosphorylated, researchers can associate particular signaling pathways with cancer initiation and progression (Mani et al 2022). They can also inhibit the phosphorylation events involved to halt cancer progression.

  The production and activity of many proteins can also be altered by the post-translational modification known as acetylation. This PTM involves the addition of a small chemical group to parts of proteins. For DNA-scaffolding proteins called histones, this can result in the DNA being more or less accessible to other proteins that read and decode it. When acetylation causes a stretch of DNA to be more accessible, the proteins in that stretch can be produced in higher amounts and vice versa. Acetylation of other proteins can also alter their activity and lead to changes in cellular function. In fact, changes in acetylation are associated with characteristics that can drive cancer, that can stratify cancer severity, and that can direct physicians to specific treatments (Harachi et al 2021, Zhang et al 2016). If researchers can use proteomics technologies to associate more acetylation events with specific cancer cell functionalities, they may also be able to design compounds that inhibit these acetylation events or modify the activities of acetylated proteins.

- A genome-agnostic assessment of changes in protein abundance. In the above examples, proteomics technologies are framed as a means to complement genomics. However, proteomics has a large role to play on its own. By comparing the proteomes of cancer cells to the healthy cells they're derived from, researchers can identify proteins with altered abundance regardless of the underlying genomic mutations. In addition, by comparing the proteomes of cancer cells from patients with varying degrees of aggressive disease, they can independently associate particular "proteomic profiles" with levels of severity. Using this information, physicians can treat their patients more or less aggressively. For instance, they might be more willing to use a treatment with particularly strong side effects if a patient has a very severe cancer and poor prognosis.

  Looking at proteomic data agnostic to genomics data can also be useful because a variety of non-genomic factors can alter cancer cell behavior. For instance, cancer cells can often resist attacks from immune cells and even produce compounds that tamp-down the immune response. Production of the proteins involved in these interactions can be induced by genetic mutations, communication with other cells, and conditions in the tumor's environment. Even when immune evasion is genetically based, the changes in protein expression that enable it won't necessarily be obvious from the initial mutations alone. Thus, by looking at changes in protein expression indicative of immune evasion instead of looking at the genome, researchers can devise ways to effectively give the immune system a boost and kill cancer cells (Hanahan and Weinberg 2011, Spranger and Gajewski 2018).

  Similarly, cancer cell interactions with the microorganisms that live on and in us, the microbiome, are increasingly being recognized as important factors in cancer progression (Hanahan 2022). Although there are not always genomic mutations associated with some of these interactions, researchers can use proteomics to look for protein-based signatures of them.

The proteome and cancer treatment

When it comes to developing new cancer treatments, proteomics will accelerate research in a number of ways. Some of these were touched upon in the previous section and include:

- Prioritizing which mutant genes to target based on the abundance of the encoded proteins. Generally it is better to target proteins produced at a higher level in cancer cells as opposed to healthy cells. This can make drugs more selectively active on cancer cells and thereby decrease side effects and toxicity.

- Identifying potential targets downstream of a driver mutation. The protein encoded in a mutated gene may be difficult to target with a drug for a variety of reasons. By inserting this mutation into healthy cells and assessing how other protein levels change as a result, researchers can identify additional therapeutic targets. E.g. proteins whose production increases after the mutated gene is added to healthy cells and which have a known association with growth signaling might make good drug targets.

- Mining broad changes in post-translational modification and protein production. If proteins have different post-translational modifications in cancer cells or are produced at drastically different levels in cancer cells compared to healthy cells, they may be involved in the molecular mechanisms driving the cancer. Researchers can thus design drugs to alter these post-translational modifications or change the production of the aberrant proteins to determine if cancer cell growth is affected. If so, drugs targeting these modifications or proteins can be prioritized for testing in patients.

Once a therapeutic target has been established and validated through the clinical trial process, proteomics can continue to help doctors achieve more effective treatments. Proteomics can help doctors identify patients in which treatments are working well, in which cancer cells appear to be developing drug resistance, and in which treatments are failing.

To understand how this is useful, it's informative to look at one of the most effective precision medicines developed to date, the anti-cancer drug, Imatinib (Hochhaus 2017). This drug was developed to treat a cancer of the immune system called "Chronic Myeloid Leukemia" or CML. As discussed in the introduciton, CML manifests when a translocation between 2 chromosomes creates a fusion of the genes bcr and abl. The BCR-ABL fusion protein encoded in this gene continuously signals for cells to grow and drives cancer progression.

Upon discovering the causal role of BCR-ABL in CML, researchers set out to develop drugs that could inhibit its activity. They searched through a library of chemicals to find compounds that did just that and, after finding a promising hit, optimized this compound to be more easily delivered to the body and to act more selectively on BCR-ABL and not other, similar proteins. Imatinib was the result and, although it takes months for its effects to be achieved, many patients respond incredibly well to this precisely targeted drug (Sacha 2014, Hochhaus et al 2017).

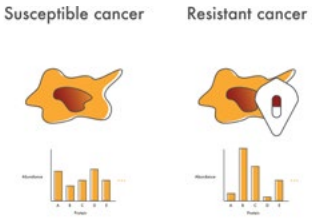
Unfortunately, Imatinib does not work in all patients. Some of them have cancer cells that produce "efflux pump" proteins which pump the drug out before it can have beneficial impacts (Illmer et al 2004). Others have additional mutations in the bcr-abl fusion gene that prevent imatinib from interacting with the fusion protein productively and still others have mutations that increase production of the BCR-ABL protein (Hochhaus et al 2002, Osman and Deininger 2021).

Researchers have created Imatinib successors that retain their activity in the face of many of these resistance mechanisms. However, many years of additional research were required to elucidate the mechanisms of resistance and optimize these newer drugs. Even so, some of these newer compounds have stronger side-effects than Imatinib and may be risky for some subgroups of CML patients to take (Osman and Deininger 2021). In addition, Imatinib and its successors are only effective during the early, less aggressive stages of CML. If patients advance beyond this stage, drug resistance is common and further progression is often BCR-ABL independent because the cancer cells acquire additional driver mutations. Finally, even when successfully treated, most patients must keep taking Imatinib or one of its successors for life to avoid relapse. This can be very expensive and efforts are ongoing to determine what distinguishes patients who don't relapse from those who do.

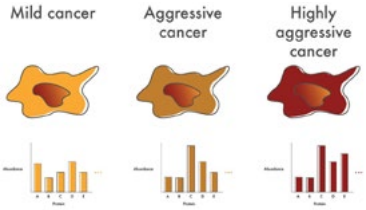
Given the above issues, even in the face of tremendous success, proteomics can accelerate and enhance the development of precision medicines like Imatinib in a number of ways. These include:

Researchers can use proteomics to look for changes in protein abundance associated with adverse reactions to new cancer drugs. These changes in abundance may indicate that the drugs being tested are not selective enough for the pathway researchers would like to target. For example, they might see drastic changes to proteins involved in heart health. These could indicate that the tested drugs will cause cardiac issues and researchers could make efforts to alter the drugs so they impact these proteins less.

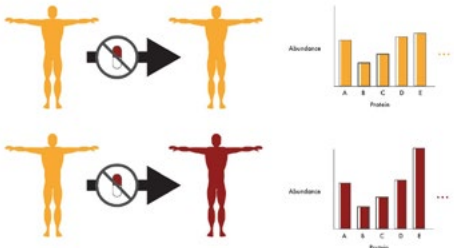
In the clinic, proteomics may give physicians early warning signs that a patient's cancer is developing resistance to a drug like Imatinib. They might, for instance, see an increase in proteins associated with cancer cell growth. As a result, they could swiftly take action to get such patients on a second line drug before the cancer returns in full force and starts having strong impacts on patient health.



In patients who do develop resistance, cancer cell proteome measurements could provide clues as to the cause of resistance. Even if they don't know what mutations are involved, researchers might see increased levels of driver proteins like BCR-ABL or increases in other proteins like the efflux proteins discussed above. With this information in hand, researchers could direct additional studies to investigate these possible sources of resistance and accelerate the development of alternative therapeutics.



In cancers like CML where advanced disease is refractory to precisely targeted drugs, researchers can use proteomics to determine what additional pathways are up-regulated in the more aggressive cancer cells. They might, for instance, look for increased phosphorylation of signaling proteins and, if they find an increase, take steps to inhibit the signaling proteins involved to stop cancer progression.



Researchers can use proteomics to look for differences in protein abundance between patients who stay in remission after they are taken off a precision medicine and those who relapse. By associating particular protein abundances with relapse, physicians could stratify patients who are good or bad candidates for being taken off the precision medicine. They could also develop new treatments that increase the production or activity of proteins associated with remission after ending therapy. These efforts could get patients off of the anti-cancer drugs faster with less risk of relapse. This would lower expenses and prevent patients from experiencing the side effects associated with these drugs.

Proteomics and cancer - a healthier outlook for the future

Proteomics should make it possible to more quickly identify the molecular causes of various types of cancer, stratify patients by disease severity to prioritize treatment, and improve clinical outcomes. As physicians and researchers use advanced proteomics technologies to catalog the proteomes of more and more cancers, they'll be able to create new protein-based diagnostic tools, identify molecular targets for therapeutics, understand mechanisms of drug resistance, and monitor therapeutic effectiveness. With the wide availability of proteomics data, there's a much brighter future in store for many cancer patients.

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