Response Paper

Noncoding regions are the main source of targetable tumor-specific antigens

Laumont et al. Sci. Transl. Med. 10, eaau5516 (2018)

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Summary

In this article, the authors propose a new workflow to identify neoantigens.
Neonatigens are cancer specific peptides (chains of up to 11 amino acids), that are presented on the cell's surface via so called HLA binding molecules to the immune system. Neoantigens are considered important targets for personalized cancer immunotherapy.

While neoantigens are mostly predicted computationally, based on patient derived next generation sequencing data, such as DNA, or RNA sequencing, these predictions often lack experimental validation and suffer from high rates of false positives.

Laumont et al. proposes a new workflow including Mass spectrometry (MS). MS is a highly sensitive device to identify peptides bound to the cell surface. The authors combined the prediction of a computational pipeline with experimental MS validation and were able to identify cancer specific neoantigens in different cancer types.

However, what is considered cancer-specific is dependent on a reference.
Traditionally, a healthy control sample from the same patient has been used as a reference point to identify tumor specific sequences. Other approaches combine large amounts of healthy tissue data, also from other patients, to build a healthy control database.
Laumont et al. use an innovative approach, by using sequencing data from thymic epithelial cells (TECs).

TECs are a type of cell found in the thymus, an organ in the body that plays a crucial role in the development of T-cells. TEC cells have been shown to express numerous tissue-specific peptides to test T-cells for their self-reactiveness (self-reactive T-cells are removed), making them interesting candidates for filtering out self-peptides in cancer samples. Sequencing data from TECs can help filter for tumor specific peptides without the need for large amounts of healthy control samples from different tissues.

Another interesting finding of Laumont et al. is that the majority of their identified neonatigens came from so-called non-coding regions, which are regions of DNA that are usually not involved in the generation of proteins and peptides. However, in a cancer set up, this is indeed possible.

Neoantigens derived from non-coding regions have been proposed as a new target population for immunotherapy, whose potential has yet to be uncovered.

Reflection and thoughts

This article is certainly difficult to understand for someone not really familiar with cancer immunotherapy and neoantigens. This not only includes laymen, but also biologists, or doctors, who haven't heard of neoantigens.

However, most people know that for transplantation a close match must be found, otherwise the transplanted tissue gets rejected by the immune system of the recipient.
This is due to person-specific peptides that are presented on the cell's surface to the immune system, that act something like an ID, that states "This cell belongs to this body, immune system, please don't kill it!".

Close relatives often have a similar landscape of these presented peptides, since they share the DNA regions determining how this peptide landscape looks like.

If a virus enters a cell and enslaves it to produce viral proteins, the resulting viral peptides also get presented via the cell surface to the immune system. These viral peptides are considered "foreign", or "non-self" by the immune system, T cells then proceed to eliminate the infected cell.

A similar principle holds true for cancer cells. Cancer DNA is often mutated, if this mutated DNA is made into a protein, it will also lead to mutated peptides, which again, will be presented to the immune system. Although it is the same cell, these mutations can still be recognized as foreign and the cancer cell gets killed.
These mutated peptides, also referred to as Neoantigens, are tumor specific and are not present on healthy cells, this makes them ideal targets for therapy.

While the immune system normally takes care of the cancer cells, they can evade this mechanism in various ways, T cells can get exhausted, or cancer cells simply grow faster than they can be killed. Immunotherapy seeks to support the patient's own immune system to kill cancer cells presenting neoantigens.

Different Immunotherapy approaches exist, however they all have in common that they are dependent on neoantigens. For some of the therapies, such as cancer vaccination, the neoantigen must be known to design a vaccine based on it. The vaccine increases the T cell population reactive to the neoantigens and might skew the battle in favor of the immune system.

In recent years, many bioinformatic pipelines have been developed to identify neoantigens from a patient's individual DNA, or RNA sequencing data.
Despite the numerous challenges in reconstructing such complex biological processes, pipelines are getting more accurate and efficient. However, there is another, general problem with Immunotherapies.

Since neoantigens come from mutations in the DNA, the total number of such mutations has proven to be a decent predictor for therapy success.
However, not every cancer type has the same amount of mutations, there are cancer types, such as skin cancer, with a naturally high number of mutations and decent treatment

success, while gut cancer, with usually a low number of mutations, shows little to none response to Immunotherapy. Cancer with low numbers of mutations make up the majority of cancer types.

Laumont et al., but also other papers, have proven that there is a large potential in neoantigens that are more independent of the overall number of mutations. These neoantigens are often referred to as non-canonical neoantigens (ncnas), since they derive from sources other than single mutations in the DNA.
In cancer cells many genetic abbreviations can occur, for example two proteins can get fused together, dormant viral RNA can get reactivated, or transcripts can get heavily edited. All these abbreviations can lead to non-canonical neoantigens.

While there are many prediction pipelines present for the prediction of canonical neoantigens (derived from single mutations), pipelines for ncna prediction are currently sparse. Since the sources for ncnas are way broader, their prediction is more complex, there is currently a lack in common workflows for this type of analysis.

To really understand how important ncnas can be for immunotherapy and patients with low mutation cancers, new pipelines are needed.

The workflow presented by Laumont et al. is definitely powerful and comprehensive, however MS validation is tedious and expensive, especially considering a larger patient cohort. There is a need for standalone computational pipelines overcoming current limitations to ultimately aid in large scale therapy studies.

What makes Laumont et al.'s work also noteworthy, is their innovative use of TECs for filtering, which might make large filtering databases obsolete.

In my pipeline, which will be called NovumRNA, I will use the same data for filtering, however, the user can extend the database with their own data.
NovumRNA seeks to overcome current limitations and be the first comprehensive ncna prediction pipeline allowing for large-scale identification of ncans in different cancer types to further understand their potential in cancer immunotherapy.