Uncovering unknown TUMOR ANTIGENS

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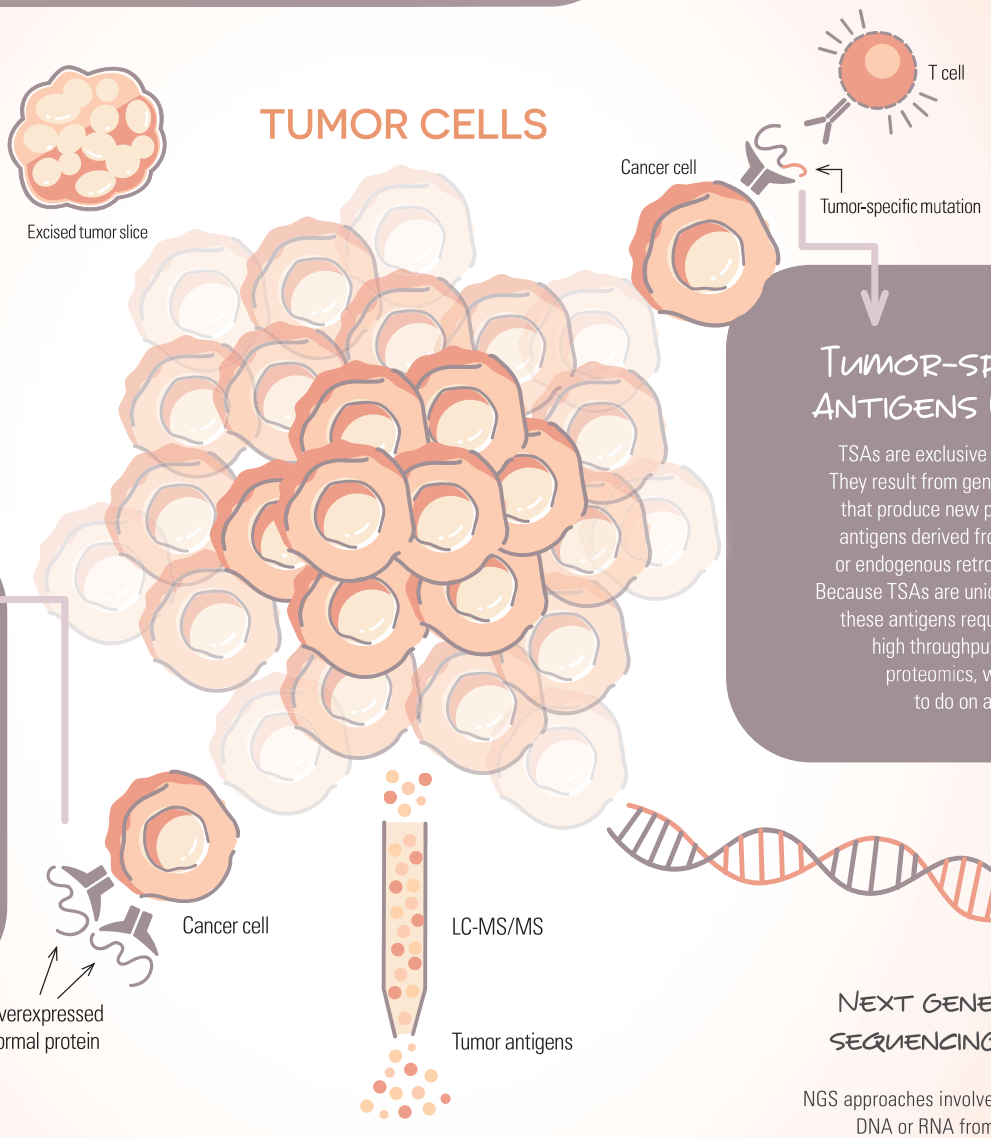
Tumor antigens are proteins or molecules presented on the surface of tumor cells by the major histocompatibility complex (MHC) that stimulate an immune response in the body. Detecting tumor antigens is key to developing effective cancer diagnostics, immunotherapies, and vaccines. Many antigens that define specific cancer types at the population and individual levels remain a mystery, spurring researchers to hunt for more effective ways to identify

Complimentary DNA (cDNA) expression library screening

cDNA expression library screening involves isolating total RNA from tumor cells and converting it into cDNA plasmids that are then transfected into cell lines in culture. Researchers then coculture the cells with isolated tumor reactive T cells and screen for which cDNA plasmids elicit a T cell response (2).

Unbiased tumor antigen screening

With unbiased tumor antigen screening, researchers perform whole exome sequencing on excised tumors to identify single nucleotide variants and small genomic insertions and deletions. They then create pooled antigen libraries and pulse them into antigen-presenting cells, thereby exposing the antigens to all possible MHC molecules. They then coculture the cells with tumor infiltrating-lymphocytes to detect which antigen pools elicit T cell activation (2).



Tumor-specific antigens (TSAs)

TSAs are exclusive to cancer cells. They result from genetic alterations that produce new proteins or from antigens derived from oncoviruses or endogenous retroviral elements. Because TSAs are unique, identifying these antigens requires combining high throughput genomics and proteomics, which is difficult to do on a large scale (1).

Tumor-associated antigens (TAAs)

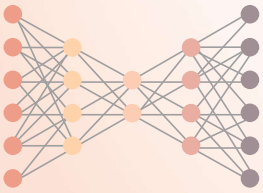
TAAs are normal proteins, such as germline proteins, that are overexpressed in cancer cells. Because TAAs are also expressed in normal tissues, immunotherapies targeting TAAs may not elicit effective antitumor responses and pose the risk of inducing autoimmunity (1).

Next generation sequencing (NGS)

NGS approaches involve sequencing DNA or RNA from normal and cancerous tissue and using bioinformatic algorithms to identify altered genomic regions. However, some NGS approaches such as whole exome sequencing may miss alternatively spliced TSAs (3). As such, NGS is best paired with immunopeptidomes approaches (2).

Immunopeptidomics

Immunopeptidomics involves eluting antigens from their complexes with MHC molecules and running them through liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) columns. To identify tumor antigens, researchers then compare the LC-MS/MS spectra to custom databases containing NGS data from tumors with reference protein sequences. While LC-MS/MS is better at capturing post-translational modifications, it has limited sensitivity and several biases (2).



Prediction algorithms

Several machine learning algorithms trained on experimental data can artificially generate antigens derived from different proteins and predict their ability to bind specific MHC molecules that would trigger T cell responses. However, it is important to experimentally validate algorithm predictions for immunogenicity (2).

REFERENCES

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