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IMMUNOTHERAPY

Expanding the search

Few actionable tumour-specific antigens (TSAs) successfully used in therapeutic cancer vaccines have been discovered so far. Most efforts to date have employed the approach of reverse immunology to identify mutations from whole-exome sequencing of tumour cells, and major histocompatibility complex (MHC)-binding prediction software algorithms to assess the binding affinities of mutated peptides. Yet, this method has drawbacks: 90% of TSA candidates are false positives, as the software tools fail to predict steps other than binding necessary for the processing of MHC peptides, and using only protein-coding exons as a source of TSAs is limiting. To address this, Laumont, Vincent et al. developed a proteogenomic strategy for high-throughput mass spectrometry (MS) discovery of aberrantly expressed TSAs (aeTSAs) from non-coding sequences as well as mutated TSAs (mTSAs) encoded by all genomic regions.

Most MS software tools rely on a user-defined reference proteome that does not include TSAs, and so the authors built a tailored database from tumour RNA sequencing data. This global cancer database comprises a canonical cancer proteome of proteins encoded by normal or single base-mutated exonic sequences, and a cancer-specific proteome of peptides encoded by any reading frame of any genomic region that are absent from normal MHC II^{hi} medullary thymic epithelial cells (mTECs, which are responsible for T cell selection).

Utilizing the database with MS sequencing of two mouse tumour cell lines, colorectal carcinoma CT26 and T lymphoblastic lymphoma EL4, followed by further validation identified 6 mTSAs and 11 aeTSAs. Interestingly, most of the TSAs came from atypical translation events: translation of an out-of-frame coding exon or non-coding regions. Furthermore, these TSAs derived from various non-coding regions, such as intergenic and intronic sequences, non-coding exons, untranslated region (UTR)–exon junctions, endogenous retroelements and even structural variants.

Double immunization of mice with either unpulsed or individual TSA-pulsed dendritic cells before challenge with live EL4 cells demonstrated that the identified TSAs displayed differential but overall long-lasting protection. Crucially, the immunogenicity of the TSAs was determined by both TSA-responsive T cell expansions upon vaccination and TSA expression levels on tumour cells.

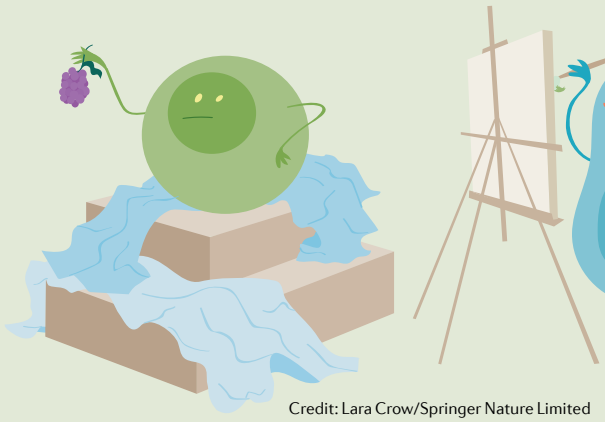
> “targetable antigens that can be used for cancer vaccine development”

TUMOUR IMMUNOLOGY

Organoid 2.0

One shortcoming of organoid culture is the lack of stroma, blood vessels and immune cells constituting the tumour microenvironment (TME). To date, the development of co-culture systems of primary tumour epithelia incorporating additional cellular components, such as tumour-infiltrating lymphocytes (TILs), without artificial reconstitution has proven challenging. Now, Neal, Li et al. have successfully established patient-derived organoids (PDOs) of tumour epithelia retaining native immune cells, thereby recapitulating TME diversity.

To model the immune TME in 3D, the authors adapted their previously reported air–liquid interface (ALI) method for culturing mouse organoids to propagate human biopsy samples as PDOs. Surgically resected primary and metastatic tumours were used to establish PDOs from 100 individual patient tumours, totalling 14 different tissue sites and 28 disease subtypes. Mechanically dissociated tumour fragments were plated, expanded and serially passaged in a type I collagen matrix with a WNT3A, epithelial growth factor (EGF), noggin and R-spondin 1-based culture medium. These organoids could be maintained long term, cryopreserved without substantial loss of viability and xenografted into immunocompromised mice and re-derived as organoids.


Credit: Lara Crow/Springer Nature Limited

> “patient-derived organoids (PDOs) of tumour epithelia retaining native immune cells”

PDOs were generated from both common cancers, such as colon, and less frequently occurring cancers, such as brain schwannoma. Both genetically and phenotypically, PDOs resembled the tumour epithelium from which they were derived. Expected genetic alterations were identified: for example, adenomatous polyposis coli (*APC*) loss in colorectal adenocarcinoma organoids. Furthermore, the stroma of the PDOs contained myofibroblasts in close proximity with the tumour epithelium.

Importantly, immune cell populations were also preserved in the PDOs with infiltrating CD3$^+$ T cells expressing programmed cell death protein 1 (PD1) tightly associated with the tumour epithelium. Cytotoxic T cells, T helper cells, B cells, natural killer (NK) cells, NKT cells and variable numbers of macrophages were also detected.

Next, the authors developed a droplet-based assay called the chromium immune profiling solution to simultaneously assess gene expression,

Last, applying this strategy to human primary tumour samples — specifically four B cell acute lymphoblastic leukaemias (B-ALLs) and three lung cancers — by subtracting mRNA sequences of TECs and mTECs from unrelated donors led to the identification of 2 mTSAs and 20 aeTSAs. One of the B-ALL aeTSAs originates from the 3′ UTR of *TCL1A*, a gene associated with lymphoid malignancies. The next step would be to validate the immunogenicity of these TSAs in mice.

By identifying TSAs from non-coding regions, this study expands the number of targetable antigens that can be used for cancer vaccine development. Even more appealing as preferred targets, aeTSAs can be widely shared by multiple tumours (including those with low mutational burdens), meaning a single vaccine could be generated, unlike neoantigen-directed immunotherapy, which is likely to be personalized.

Anna Dart

ORIGINAL ARTICLE Laumont, C. M., Vincent, K. et al. Noncoding regions are the main source of targetable tumor-specific antigens. *Sci. Transl Med.* **10**, eaau5516 (2018)

T cell receptor (TCR) and B cell immunoglobulin repertoires from single cells of the same sample. This revealed that immune cell subsets, including exhausted T cells, and TCR clones were conserved between matched fresh tumours and organoids.

Last, to determine TIL functionality in these models, 20 further PDOs were generated from immunotherapy-responsive tumours, such as melanoma. Treatment with the PD1 antibody nivolumab resulted in induction of activation markers in expanding $CD3^+$ T cells and tumour cell killing in 6 of the 20 PDOs, indicative of response to immune checkpoint inhibition and consistent with clinical trial data.

This organoid method, preserving the immune contexture, has great promise for in vitro modelling of personalized immunotherapy.

Anna Dart

ORIGINAL ARTICLE Neal, J. T. et al. Organoid modeling of the tumor immune microenvironment. *Cell* **175**, 1972–1988 (2018)
FURTHER READING Drost, J. & Clevers, H. Organoids in cancer research. *Nat. Rev. Cancer* **18**, 407–418 (2018)

METASTASIS

Trafficking signals for metastasis

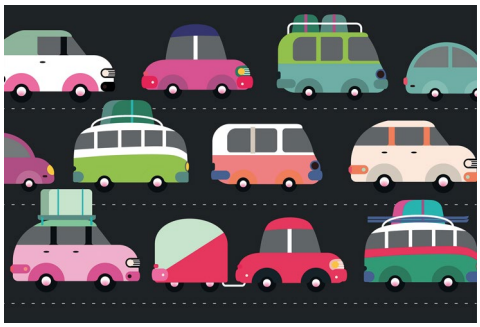
Cytotoxic chemotherapy can effectively treat invasive breast cancer, but studies in mice have suggested that it can also have pro-metastatic effects. Keklikoglou et al. now show that, in mouse models of chemoresistant breast cancer, paclitaxel and doxorubicin trigger the production of tumour-derived extracellular vesicles (EVs) with pro-metastatic properties.

The authors first treated mammary tumour-bearing mice with paclitaxel, observing that it only modestly inhibited primary tumour growth. However, compared with vehicle, paclitaxel increased the incidence and size of lung metastases or the pulmonary seeding of mammary cancer cells, depending on the mouse model.

EVs can aid the metastasis of primary tumours by influencing cells associated with the pre-metastatic niche. Here, compared with controls, EVs isolated from 4T1 mammary tumour cells (4T1 cells) or murine mammary tumours following paclitaxel treatment increased the number of metastatic nodules in tumour-free mice challenged with metastasis-forming cancer cells. Thus, paclitaxel-induced EVs appear to promote breast cancer metastasis. As EVs derived from murine mammary tumours following doxorubicin treatment also enhanced lung metastasis in mice, this phenomenon is not paclitaxel-specific.

In assessing how chemotherapy-induced EVs promote breast cancer metastasis the authors first observed that paclitaxel increased the release of EVs from mouse and human cancer cell lines as well as the number of EVs in the blood of mice with 4T1 cell-derived tumours. Furthermore, proteomic analysis revealed that EVs derived from paclitaxel-treated 4T1 cells contained higher levels of the Ca^{2+}-binding membrane-associated protein annexin A6 than EVs from vehicle-treated 4T1 cells. Paclitaxel and doxorubicin promoted the loading of annexin A6 into EVs from tumour-derived (but not non-transformed) cells, and annexin A6-deficient 4T1 cells did not promote lung colonization in mice in response to paclitaxel or doxorubicin. Thus, chemotherapy-induced EVs require annexin A6 to promote lung metastasis.

CC-chemokine ligand 2 (CCL2) can reportedly enhance breast cancer metastasis that is assisted by lymphocyte antigen 6C $(LY6C)^+$CC-chemokine receptor 2 $(CCR2)^+$ monocytes. In line with this theory, treatment of tumour-bearing (but not tumour-free) mice with paclitaxel increased the expression of CCL2 and the number of $LY6C^+$ monocytes in the lungs. EVs isolated from paclitaxel-treated mice with tumours also increased the abundance of $LY6C^+CCR2^+$ monocytes in the lungs of tumour-free mice. As paclitaxel-induced EVs did not promote pulmonary metastasis in *Ccr2*-knockout mice or increase the expression of *Ccl2* or *Ccr2*, or the number of $LY6C^+$ monocytes, in the lungs of annexin A6-null mice, annexin A6 may initiate metastasis by expanding $LY6C^+CCR2^+$ monocytes.



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Finally, looking at the biology of cells in the lung pre-metastatic niche, the authors observed that paclitaxel promoted the internalization of EVs by lung endothelial cells. By using murine endothelial bEnd.3 cells, the authors found that annexin A6 was transferred from paclitaxel-induced EVs to bEnd.3 cells, where it co-localized with the nuclear factor-κB (NF-κB) subunit p65. NF-κB can activate the transcription of *Ccl2* and, here, paclitaxel-induced EVs increased NF-κB activity and *Ccl2* expression in bEnd.3 cells in an annexin A6-dependent manner.

Taking all of their data into account, the authors propose that chemotherapy-induced EVs transfer annexin A6 to pulmonary endothelial cells, where it promotes the upregulation of CCL2 and the accumulation of $LY6C^+$ monocytes to allow tumours to colonize at metastatic sites. As annexin A6 levels were increased in the plasma of patients with breast cancer undergoing neoadjuvant chemotherapy, chemotherapy-induced EVs are potential biomarkers of pulmonary metastasis.

Katharine H. Wrighton

ORIGINAL ARTICLE Keklikoglou, I. et al. Chemotherapy elicits pro-metastatic extracellular vesicles in breast cancer models. *Nat. Cell Biol.* https://doi.org/10.1038/s41556-018-0256-3 (2018)