Organoid-Based Immunopeptidomics Reveals Pancreatic Cancer–Restricted Cryptic Antigens

Background and Rationale

Pancreatic ductal adenocarcinoma (PDAC) remains one of the most lethal malignancies, characterized by poor responsiveness to immune checkpoint blockade and other T cell–based immunotherapies. A defining feature of PDAC is its low-to-intermediate tumor mutational burden, which severely limits the availability of classical mutation-derived neoantigens.

This biological reality raises a fundamental methodological question: *Are mutation-derived neoantigens the correct antigenic space to interrogate in low-mutational cancers such as PDAC?*

A recent **Science** study proposes a decisive shift in strategy. Rather than inferring classical neoantigens, the authors directly interrogated what tumor cells actually present to T cells, using immunopeptidomics applied to patient-derived pancreatic cancer organoids.

Immunopeptidomics as a Direct Readout of Antigen Presentation

Immunopeptidomics focuses on the immunopeptidome—the complete repertoire of peptides bound to major histocompatibility complex (MHC) molecules (HLA in humans) and displayed on the cell surface.

From an immunological perspective, this represents the *effective antigenic landscape* available for T cell surveillance. Unlike genomic or transcriptomic analyses, immunopeptidomics captures:

- Antigen processing and proteasomal cleavage
- Peptide transport and MHC loading
- Stability and surface presentation of peptide–MHC complexes

Thus, it directly answers a mechanistic question central to T cell immunity: *Which peptides are physically presented by tumor cells and therefore accessible to TCR recognition?*

In this study, HLA class I–associated peptides were isolated by immunoaffinity purification of intact HLA–peptide complexes, followed by LC–MS/MS–based peptide identification. This workflow avoids reliance on binding prediction alone and enables unbiased discovery of presented antigens, including those derived from non-canonical genomic regions.

Patient-Derived Organoids as a Signal-Enrichment Strategy

Bulk pancreatic tumor tissue is dominated by stromal components, often diluting tumor-derived signals. As a result, immunopeptidomic analyses of bulk samples can underestimate or entirely miss tumor-restricted antigens.

To address this, the authors established patient-derived organoids (PDOs) from PDAC samples. This approach enriches for malignant epithelial cells while preserving patient-specific genetic and epigenetic features.

Immunopeptidomic analysis of 11 HLA class I–positive PDOs yielded >90,000 unique HLA-I–associated peptides, with 8,000–18,500 peptides per patient.

Integration with single-cell RNA sequencing and peptide source gene module (PSGM) analysis demonstrated that PDO-derived immunopeptidomes were strongly enriched for malignant cell–specific signatures compared with bulk tumor material.

Discovery of Non-Canonical, Cancer-Restricted Antigens

Consistent with prior knowledge, only five mutation-derived HLA peptides were detected across four PDAC samples, underscoring the limited applicability of classical neoantigen paradigms in this disease.

In contrast, the authors identified more than 1,700 non-canonical HLA-associated peptides (ncHLAp) originating from non-coding genomic regions, including:

- Long non-coding RNAs
- Untranslated regions (5′ and 3′ UTRs)
- Alternative and internal open reading frames

Among these, over 500 peptides were classified as pancreatic cancer–restricted cryptic antigens (CR ncHLAp), absent from normal tissue datasets.

Importantly, 29% of CR ncHLAp were shared across multiple patients, suggesting a degree of inter-patient convergence not typically observed for mutation-derived neoantigens.

Functional Validation: Establishing HLA-Dependent T Cell Recognition

A central strength of this study lies in its transition from antigen discovery to mechanistic validation.

A subset of CR ncHLAp was evaluated in functional assays, revealing that 36.3% induced cytotoxic T cell activation in vitro.

To directly test whether T cell recognition was mediated through HLA class I, the authors employed *InVivo*MAb™ anti-human MHC Class I antibody (clone W6/32, Cat. #BE0079; Bio X Cell).

PDOs were pre-incubated with W6/32 antibody (50 µg/mL, 30 min) prior to co-culture with antigen-specific TCR-engineered T cells. Blocking surface HLA-I molecules significantly reduced tumor cell killing, establishing that:

- Cryptic antigens are presented via HLA class I
- T cell–mediated cytotoxicity is strictly HLA-I–dependent

This experiment provides causal evidence linking cryptic antigen presentation to functional T cell recognition.

Implications for Immunotherapy Development

This work redefines antigen discovery in low-mutational cancers by demonstrating that:

- The immunopeptidome extends far beyond canonical coding regions
- Cryptic antigens can be both tumor-restricted and immunogenic
- Organoid-based immunopeptidomics enables high-confidence antigen discovery and validation

From a translational perspective, these findings support the development of TCR-engineered therapies and cancer vaccines targeting non-canonical antigens that are otherwise inaccessible through genomics-driven pipelines.

Antibody Considerations for Immunopeptidomics Workflows

High-resolution immunopeptidomics and downstream functional validation place stringent demands on antibody quality. Bio X Cell provides functional-grade, preservative-free, low-endotoxin antibodies optimized for:

- HLA immunoprecipitation
- Surface blocking in T cell co-culture assays
- Organoid and in vivo–relevant experimental systems

Commonly used reagents include:

- **BE0079** - *InVivo*MAb anti-human MHC Class I (HLA-A, HLA-B, HLA-C)
- **BE0469** - *InVivo*MAb anti-human HLA-A2
- **BE0483** - *InVivo*MAb anti-human HLA-E

- **BE0452** - *InVivo*MAb anti-human pan MHC Class II (HLA-II)

Concluding Perspective

By integrating organoid biology with immunopeptidomics and functional immunology, this study establishes a rigorous framework for antigen discovery in cancers previously considered immunologically intractable. As the field moves toward non-canonical antigen spaces, methodological precision—particularly in antigen isolation and functional validation—will be decisive.

References

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