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Beyond Traditional Biomarkers: Exploring the Application of Serum Peptide Profiling in Enhancing Tumor Diagnostic Efficiency

In the field of tumor diagnosis and treatment, early and precise diagnosis is crucial for improving patient prognosis. With the rapid advancement of proteomics technology, serum peptides—these "molecular fingerprints" originating from the tumor microenvironment and systemic pathophysiological processes—are emerging as the core of a new generation of liquid biopsy biomarkers. Analytical technologies based on high-resolution mass spectrometry and artificial intelligence can now decode complex peptide profiles from a single drop of blood, enabling comprehensive workflow management from high-risk population screening and early tumor diagnosis to molecular subtyping and recurrence monitoring. This paper aims to provide a systematic review of the latest research advances, core technological breakthroughs, challenges faced, and future development directions in this field.

I.From Clinical Dilemmas to Technological Breakthroughs: The Rise of Peptide Biomarkers

Current tumor diagnosis, particularly for deep-seated tumors such as those in the liver, gallbladder, and pancreas, heavily relies on imaging examinations and invasive biopsies. Traditional serum protein markers such as carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), and alpha-fetoprotein (AFP) often fall short due to insufficient sensitivity and specificity, particularly in early-stage detection and distinguishing between benign and malignant conditions, failing to meet clinical demands. For instance, CA19-9 levels may also be elevated in benign conditions like pancreatitis, while AFP shows limited accuracy in diagnosing hepatocellular carcinoma (HCC) against the backdrop of liver cirrhosis.

Peptides offer unique advantages as biomarkers: as stable fragments produced by specific enzymatic cleavage of proteins, they provide a more direct and sensitive reflection of the activity status of proteases (such as tumor-associated matrix metalloproteinases) within the body, thereby revealing the processes of tumorigenesis, invasion, and metastasis. Compared to intact proteins, peptides exhibit greater stability in blood, and their mass spectra (peptide mass fingerprints, PMFs) demonstrate high individual and disease specificity. Consequently, systematic analysis of serum peptidomes provides a revolutionary tool for non-invasive, real-time monitoring of tumor dynamics.

II. Core Technology Advancements: From Mass Spectrometry Detection

to Intelligent Analysis.

In recent years, breakthroughs in peptide biomarker research have been closely dependent on the synergistic development of two key technological pillars: “high-dimensional data acquisition” and “intelligent information analysis.”

2.1 High-Precision Peptide Spectrum Acquisition Technology

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) is currently the mainstream platform for obtaining serum peptide profiles. It enables rapid, high-throughput detection of thousands of polypeptide feature peaks in serum (typically within the mass-to-charge ratio m/z range of 1000–4000), generating PMFs containing vast amounts of information. For instance, a study on hepatopancreatic (HPB) tumors analyzed 1,100 serum peptide features in a single run using MALDI-TOF MS. For in-depth investigations requiring higher sequence resolution, liquid chromatography-tandem mass spectrometry (LC-MS/MS) becomes an indispensable tool for identifying the precise amino acid sequences of key differentially expressed peptides.

2.2 Enzyme-Based Functionalized Probe Technology

Beyond passive detection of endogenous peptides, novel active probe technologies are pioneering an alternative approach. These techniques directly “sense” enzyme activity in serum by designing synthetic substrate peptides that can be cleaved by specific tumor-associated proteases, such

as matrix metalloproteinases (MMPs). For instance, the PAC-MANN technology for pancreatic cancer screening employs MMP2-sensitive magnetic nanopeptide probes. Requiring only 8μL of serum, it completes detection within 45 minutes. Its area under the curve (AUC) for distinguishing pancreatic ductal adenocarcinoma (PDAC) from healthy controls reaches 0.91, outperforming traditional CA19-9 detection (AUC 0.77). Combining both tests further elevates the AUC to 0.94.

2.3 Artificial Intelligence-Driven Data Analysis Model

Confronted with the ultra-high-dimensional, high-noise data generated by mass spectrometry, traditional biostatistical methods have proven inadequate. The introduction of machine learning and artificial intelligence algorithms has enabled a leap from “data” to “diagnostic decision-making.” Algorithms such as Support Vector Machines (SVM) and Random Forests (RF) can screen hundreds of candidate peptide peaks to identify the most discriminative feature combinations, constructing high-precision classification models. A multicenter study analyzed PMF data encompassing multiple hepatobiliary and pancreatic cancers using SVM and RF models. The models achieved accuracy, AUC, and Matthews Correlation Coefficient (MCC) values exceeding 0.90 on the test set, successfully enabling precise differentiation of cancer subtypes. Furthermore, the Peptide Risk Index (RI), constructed using algorithms like logistic regression, has demonstrated exceptional screening efficacy.

III.Clinical Application Panorama: From Pan-Cancer Screening to Precision Prognosis.

Research on peptide biomarkers has rapidly progressed from methodological exploration to application validation targeting specific cancer types and clinical scenarios.

3.1 Multi-Cancer Early Detection (MCED)

The latest research approach transcends the limitations of single-cancer detection by analyzing blood-derived peptides associated with the "matrix-immune-secretory" axis, which is closely linked to the tumor microenvironment, thereby enabling early detection across multiple cancer types. These biomarkers include extracellular matrix remodeling-associated proteases (e.g., MMP12), immunomodulatory peptides (e.g., CXCL13), and peptides related to the transforming growth factor-β (TGF-β) signaling pathway. The "dual-bucket strategy"—combining high-risk secretion/matrix biomarkers with immune context markers—holds promise for enhancing sensitivity while providing clues about the tumor tissue origin.

3.2 Diagnosis and Differential Diagnosis of Key Cancers

• Pancreatic Cancer: Due to the difficulty in early diagnosis, pancreatic cancer has become a hotspot for peptide biomarker research. A 2026 study identified six pancreatic cancer-specific peptides using BLOTCHIP®-MS technology and developed a risk index (RI). This model achieved 89.3%

sensitivity, 81.7% specificity, and an AUC of 0.935 in the validation cohort, with sensitivity reaching 76.5% even in patients with stage 0/IA early-stage disease.

• Hepatocellular carcinoma (HCC): Research focuses on distinguishing HCC from cirrhosis. As a circulating biomarker reflecting tumor matrix remodeling, type III collagen cross-linked propeptide (PC3X) maintains an AROC of 0.72 for differentiating HCC from cirrhosis in patients with normal AFP levels. More importantly, PC3X and AFP demonstrate complementary value in prognostic prediction. Their combination significantly enhances predictive efficacy for progression-free survival (PFS) and overall survival (OS) (hazard ratios [HR] of 2.66 and 5.86, respectively).

• Classification of Hepatobiliary and Pancreatic Tumors: A large-scale study demonstrates that a machine learning model constructed based on 71 key peptide mass fingerprints can clearly distinguish healthy controls from hepatobiliary and pancreatic cancer patients. It achieves high-precision differentiation among subtypes such as cholangiocarcinoma (CCA), hepatocellular carcinoma (HCC), gallbladder cancer (GBC), and pancreatic ductal adenocarcinoma (PDAC), providing a molecular basis for precision treatment.

3.3 Recurrence Monitoring and Prognosis Assessment

Dynamic changes in the peptide profile can effectively predict tumor

recurrence. Studies on postoperative recurrence in cholangiocarcinoma (CCA) have demonstrated that PMF using MALDI-TOF MS can rapidly distinguish early recurrence (associated with aggressive biological behavior) from late recurrence. Further LC-MS/MS analysis identified specific peptides associated with early recurrence (e.g., those derived from ATR, POLA1, and other proteins), providing a novel tool for stratified management and intervention in high-risk patients.

IV.Challenges and Future Outlook

Despite promising prospects, serum peptide diagnostics face challenges in advancing toward large-scale clinical application: First, standardization issues—including the standardization of sample collection, preprocessing, and mass spectrometry detection workflows—are prerequisites for ensuring reproducible and comparable results. Second, bioinformatics complexity necessitates larger-scale, prospective cohort studies to validate biomarker robustness and develop more powerful, interpretable AI models. Third, cost and accessibility require advancing technical simplification and cost control, such as developing portable detection devices based on nanoparticles or microfluidic chips.

Future development will focus on: 1. Multi-omics integration: Integrating peptidomics with genomic, transcriptomic, and metabolomic data to construct more comprehensive molecular profiles of tumors. 2. Diagnostic-Therapeutic Integration: Peptides serve not only for diagnosis

but also enable the construction of targeted drug delivery systems using their specific sequences. For example, the peptide-drug conjugate AVA6000, activated by Fibroblast Activation Protein (FAP), leverages tumor microenvironment-specific proteases to release chemotherapy drugs, achieving a closed-loop diagnostic-therapeutic approach. 3. Prospective Population Screening Studies: Validate the screening efficacy of peptide biomarker combinations in real-world populations, with the ultimate goal of integrating them into routine physical examinations and cancer screening systems.

V.Conclusion

Serum peptide analysis marks the dawn of a new “molecular fingerprint” era in tumor diagnostics. A technological triangle centered on high-resolution mass spectrometry, functional peptide probes, and artificial intelligence enables unprecedented precision in deciphering tumor information embedded within blood. From pan-cancer screening to personalized prognosis, peptide biomarkers are progressively filling critical gaps in the current tumor diagnostic landscape. As technological standardization advances and large-scale clinical validation is completed, liquid biopsy technologies—represented by serum peptide analysis—are poised to reshape clinical pathways for early tumor detection and precision management, ultimately delivering substantial contributions to reducing cancer mortality rates.