

Module 4: Advanced Proteomics & Mass Spectrometry

1. Mass Spectrometry (MS) in clinical oncology

Mass spectrometry (MS) serves as the cornerstone for proteomic analysis in clinical oncology, tackling the blood proteome's extreme 10^{12} dynamic range where high-abundance proteins like albumin obscure rare cancer biomarkers at pg/mL concentrations.

Core Applications

Identification: Tandem MS/MS fragments peptides for de novo sequencing and database matching (UniProt), discovering novel isoforms undetectable by immunoassays.

Quantification: Targeted MRM/PRM transitions deliver 4-5 orders linearity with <5% CV:

- Serial monitoring of therapeutic response (e.g., PSA glycoforms)
- Pharmacodynamic shifts (RTK phosphorylation cascades)

(PRM) Parallel Reaction Monitoring

(MRM) Multiple Reaction Monitoring

PTM Characterization: Phospho-/glyco-enrichment coupled with DIA/SWATH maps thousands of sites, elucidating pathway activation:

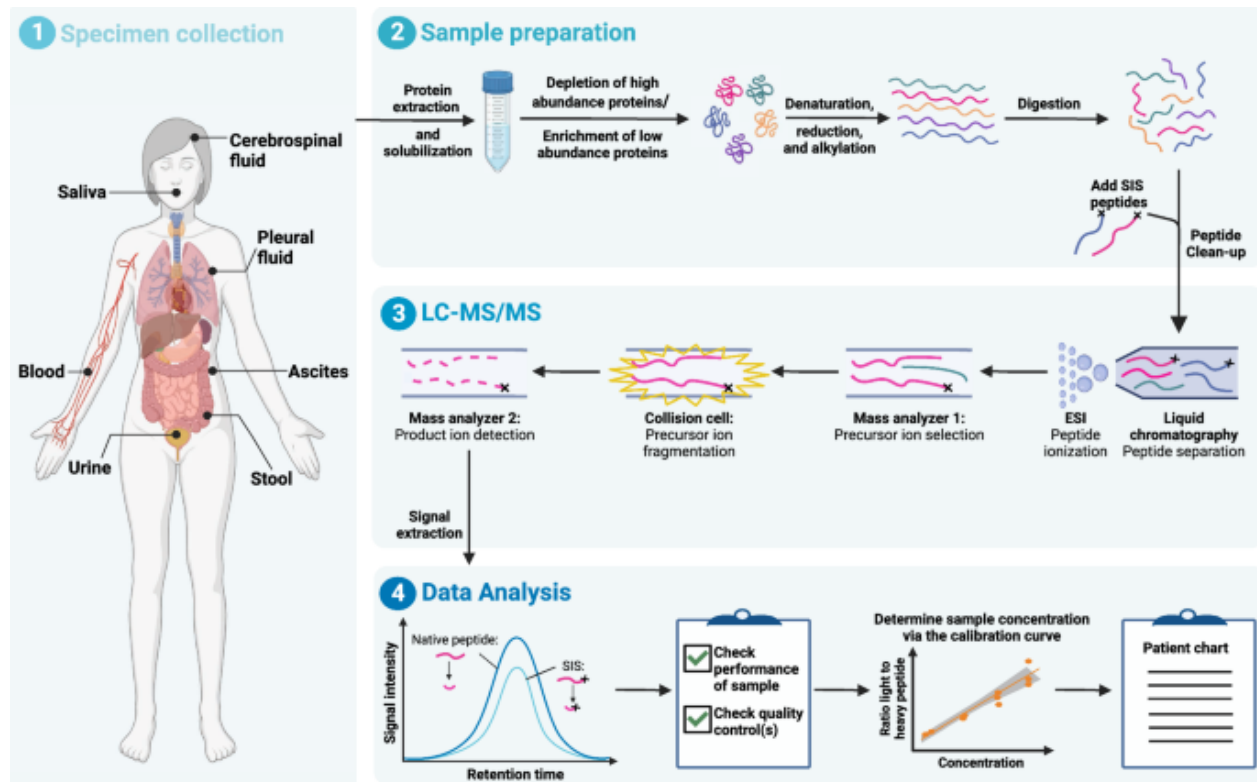


Figure 1. Typical workflow for the analysis of protein cancer biomarkers from liquid biopsies via targeted mass spectrometry (<https://link.springer.com/article/10.1186/s12014-024-09452-1>)

2. Secretomics: The study of the "Secretome"

Secretomics analyzes the complete repertoire of proteins secreted by cancer cells into the extracellular space, representing ideal circulating biomarker candidates due to direct bloodstream access (<https://www.sciencedirect.com/science/article/abs/pii/S1084952117303014?via%3Dihub>)

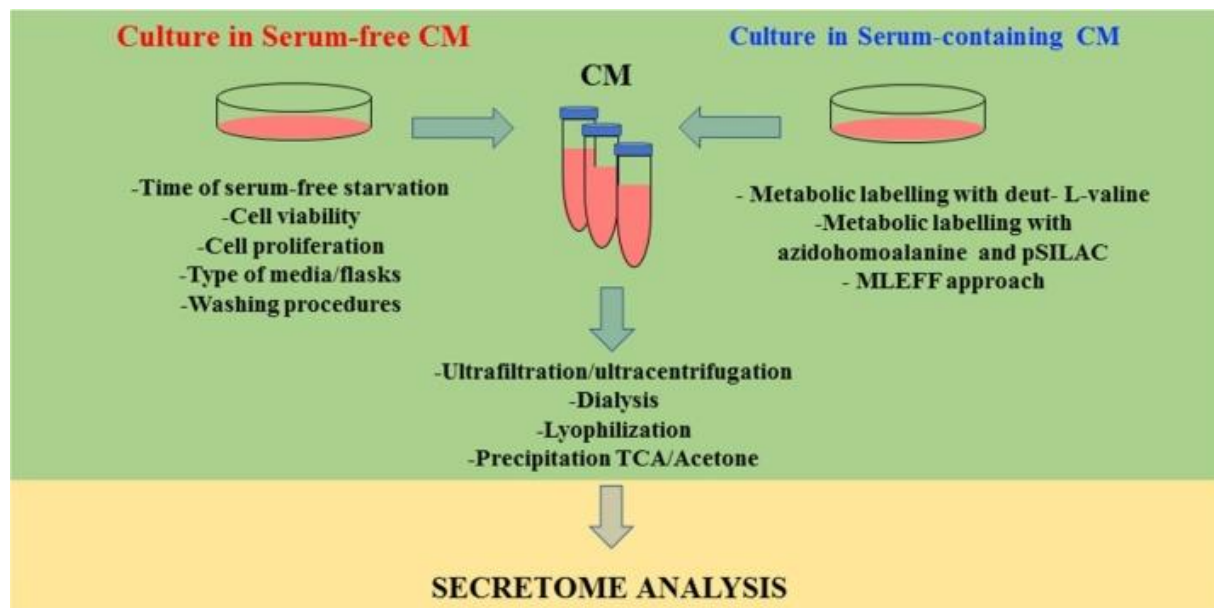


Figure 2. A representative scheme of the main aspects and strategies used to prepare the secretome sample from *in vitro* cancer cells after deprivation or in the presence of fetal bovine serum (<https://pubmed.ncbi.nlm.nih.gov/28684183/>)

Rationale: Secreted proteins bypass intracellular sampling biases and achieve detectable plasma concentrations (pg-ng/mL), unlike cytoplasmic proteins requiring cell lysis.

Secretion Pathways

Classical Secretion (~70% of secretome):

- Signal peptide → ER/Golgi → Exocytosis
- Examples: KLKs, MMPs, growth factors (PDGF, VEGF)

Non-Classical Secretion (~30%):

- Exosomal (30-150nm EVs): Lysosomal/MHC-I, cytokines
- Shedding (proteolytic ectodomain cleavage): TGF- α , HB-EGF
- ABC transporter-mediated: FGF1, IL-1 β

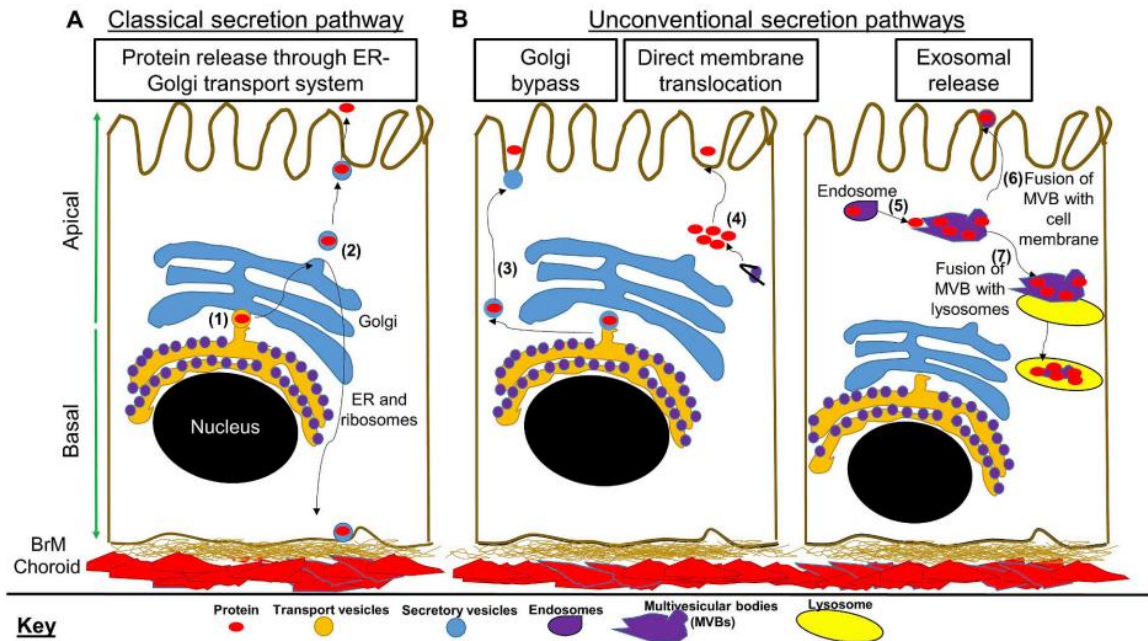


Figure 3. Cellular pathways employed by the RPE for protein secretion (<https://www.sciencedirect.com/topics/neuroscience/secretory-pathway>)

3. Quantitative Strategies: SILAC & Label-Free

To compare a "healthy" cell with a "cancer" cell, the lab uses advanced labeling techniques:

- **SILAC (Stable Isotope Labeling by Amino acids in Cell culture):**
 - One cell population is grown in "Light" amino acids, and the other in "Heavy" (stable isotope) amino acids.
 - The samples are mixed and analyzed together. The MS instrument sees two distinct peaks for the same protein, and the ratio between them tells us which state has more of that protein.
- **Label-Free Quantification (LFQ):** Compares the intensity of peptide peaks across separate MS runs. It is less expensive than SILAC and is used for large-scale clinical cohorts.

4. Bioinformatics & Pathway Analysis

Once the MS instrument generates thousands of protein IDs, bioinformatic tools (like MaxQuant or Gene Ontology) are used to:

- Categorize proteins by function (e.g., "Proteases" or "Growth Factors").
- Map proteins to signaling pathways (e.g., TGF-beta or MAPK).
- Identify "hub" proteins that could be novel therapeutic targets.

