Cancer

1) Molecular Mechanisms of Cancer Metastasis

Cancer metastasis is a complex, multistep process through which cancer cells spread from the primary tumor to distant organs. This process involves various molecular mechanisms, including genetic mutations, changes in signaling pathways, and interactions with the tumor microenvironment. The metastatic cascade consists of several key steps: **local invasion, intravasation, circulation, extravasation, and colonization**. Below is a detailed breakdown of the molecular mechanisms involved at each step.

Local Invasion (Epithelial-Mesenchymal Transition - EMT)

Before cancer cells can spread, they must first **detach from the primary tumor and invade the surrounding tissue**. This is facilitated by a process called **epithelial-mesenchymal transition (EMT)**, where epithelial cancer cells lose their adhesion properties and acquire mesenchymal characteristics, which enable motility and invasiveness.

Key Molecular Players in EMT:

- **Loss of E-Cadherin**:
    - E-cadherin, a key cell-cell adhesion protein, is often downregulated in metastatic cancer cells.
    - This loss is driven by transcriptional repressors like **Snail, Slug, ZEB1, ZEB2, and Twist**.
- **Cytoskeletal Reorganization**:
    - Actin remodeling proteins such as **Rho GTPases (RhoA, Rac1, and Cdc42)** enable cancer cells to acquire motility.
    - Focal adhesion molecules (FAK, integrins) allow cells to migrate by interacting with the extracellular matrix (ECM).
- **Matrix Degradation (MMPs)**:
    - Cancer cells secrete **matrix metalloproteinases (MMPs), such as MMP-2 and MMP-9**, which degrade the ECM, allowing cells to invade neighboring tissues.

Intravasation (Entry into the Bloodstream or Lymphatic System)

Once cancer cells have invaded local tissue, they enter **blood vessels or lymphatic vessels** in a process called intravasation.

Key Molecular Players in Intravasation:

- **Tumor-Associated Macrophages (TAMs)**:
    - TAMs secrete cytokines like **TNF-α and IL-6**, which promote inflammation and weaken endothelial barriers, facilitating cancer cell entry.
- **VEGF (Vascular Endothelial Growth Factor)**:

    - VEGF increases blood vessel permeability, allowing cancer cells to enter circulation.
- **MMPs and Cathepsins**:
    - These enzymes break down basement membranes of blood vessels, aiding in intravasation.

Circulation (Survival in the Bloodstream)

Once inside the bloodstream, circulating tumor cells (CTCs) must survive in a harsh environment where they face **immune attack, mechanical stress, and oxidative damage**.

Key Molecular Players in Circulatory Survival:

- **Platelet Cloaking**:
    - Cancer cells bind to platelets through **integrins (αIIbβ3, αvβ3)** and **TGF-β signaling**, forming a protective shield against immune attacks.
- **Anoikis Resistance**:
    - Normal epithelial cells undergo **anoikis (a form of apoptosis) when detached from the ECM**, but metastatic cells evade this by activating **PI3K/AKT and NF-κB survival pathways**.
- **Evasion of Immune Surveillance**:
    - Cancer cells downregulate **MHC I** and upregulate **PD-L1**, inhibiting T-cell recognition and attack.

Extravasation (Exit from the Bloodstream into a Secondary Site)

To colonize a new organ, cancer cells must **exit the circulation (extravasation)** and invade distant tissues.

Key Molecular Players in Extravasation:

- **Selectins (E-, P-, and L-Selectin)**:
    - These adhesion molecules on endothelial cells help cancer cells slow down and attach to blood vessel walls.
- **Integrins (α3β1, α6β4)**:
    - These molecules facilitate the attachment of CTCs to endothelial cells and help cancer cells pass through the blood vessel walls.
- **Pericytes and Endothelial Cell Interactions**:
    - VEGF and **angiopoietins (ANG1, ANG2)** disrupt endothelial junctions, making it easier for cancer cells to migrate into tissues.

Colonization (Formation of Secondary Tumors)

The final and most challenging step is **colonization**, where cancer cells must **adapt to the new microenvironment** and form a secondary tumor.

Key Molecular Players in Colonization:

- **Seed and Soil Hypothesis (Microenvironment Adaptation)**:
  - Certain cancers preferentially metastasize to specific organs due to a compatible microenvironment.
  - Example: **Breast cancer → Bone, Lung Cancer → Brain, Colon Cancer → Liver**.
- **Cancer Stem Cells (CSCs)**:
  - A small population of cancer cells exhibits **stem cell-like properties**, allowing them to self-renew and survive in a foreign environment.
- **Dormancy and Reactivation**:
  - Some cancer cells remain dormant for years before reactivating under specific conditions (e.g., stress, inflammation, angiogenesis).
- **Neoangiogenesis (New Blood Vessel Formation)**:
  - Metastatic tumors induce blood vessel growth through **VEGF, Angiopoietins, and Hypoxia-Inducible Factor (HIF-1α)** to obtain nutrients.

2) Identification of Novel Cancer Biomarkers for Early Diagnosis

Cancer biomarkers are biological molecules found in blood, tissue, or other body fluids that indicate the presence of cancer. They can be **proteins, DNA/RNA mutations, epigenetic changes, or metabolites** that signal early tumorigenesis. Identifying novel biomarkers for early cancer detection is crucial because early-stage cancers are more treatable and have higher survival rates.

Modern biomarker discovery involves **genomics, transcriptomics, proteomics, and metabolomics** using high-throughput sequencing and artificial intelligence (AI)-based data analysis.

Types of Cancer Biomarkers

Biomarkers for early diagnosis fall into different categories based on their molecular nature and detection methods.

1. Genetic Biomarkers (DNA Mutations)

Mutations or alterations in DNA can be early indicators of cancer.
Examples:

- **TP53 Mutations** Found in early-stage lung, breast, and colorectal cancers.
- **KRAS Mutations** Common in pancreatic and colorectal cancers.
- **BRCA1/BRCA2 Mutations** Linked to hereditary breast and ovarian cancer.

2. Epigenetic Biomarkers (DNA Methylation, Histone Modifications)

Epigenetic changes often occur before cancer develops, making them useful for early detection.
Examples:

- **Methylated SEPT9 (mSEPT9)** Found in early-stage colorectal cancer.
- **MGMT Promoter Methylation** Associated with glioblastomas.
- **RASSF1A Hypermethylation** Present in lung and breast cancer.

3. Circulating Tumor DNA (ctDNA) and Liquid Biopsy

Small fragments of tumor DNA released into the bloodstream can be detected for early diagnosis.
Examples:

- **EGFR Mutations in ctDNA** Used for non-small cell lung cancer detection.
- **BRAF V600E Mutations in ctDNA** Found in melanoma and colorectal cancer.

4. Protein Biomarkers

Certain proteins are overexpressed or uniquely produced by early-stage tumors.
Examples:

- **Alpha-fetoprotein (AFP)** Early biomarker for liver cancer.
- **Carcinoembryonic Antigen (CEA)** Used for colorectal and lung cancer screening.
- **Prostate-Specific Antigen (PSA)** Early detection of prostate cancer.

5. MicroRNA (miRNA) Biomarkers

MicroRNAs are small, non-coding RNAs that regulate gene expression and serve as early cancer indicators.
Examples:

- **miR-21, miR-155** Elevated in lung, breast, and pancreatic cancers.
- **miR-34a** Suppressed in early colorectal cancer.
- **miR-16, miR-195** Biomarkers for early-stage breast cancer.

6. Metabolomic Biomarkers

Cancer cells alter metabolism, producing unique metabolites detectable in biofluids.
Examples:

- **2-Hydroxyglutarate (2-HG)** Biomarker for gliomas.
- **Lactate and Choline Levels** Elevated in aggressive tumors.
- **Polyamines** Seen in early colorectal and prostate cancers.

Methods for Identifying Novel Biomarkers

Modern techniques help identify and validate new cancer biomarkers.

1. Genomic and Transcriptomic Approaches

- **Next-Generation Sequencing (NGS)** Detects cancer-associated mutations in DNA and RNA.
- **RNA-Seq** Identifies novel cancer-related mRNA and non-coding RNA expression patterns.

2. Proteomic Approaches

- **Mass Spectrometry (MS)** Identifies cancer-specific proteins in biofluids.
- **Protein Microarrays** Detects autoantibodies against tumor-associated antigens.

3. Liquid Biopsy

- **Circulating Tumor Cells (CTCs)** Isolated from blood for early cancer detection.
- **Exosome Analysis** Small vesicles carrying cancer-derived proteins and RNA can be used for diagnosis.

4. AI and Machine Learning in Biomarker Discovery

AI algorithms analyze large omics datasets to identify patterns in gene expression, mutations, and metabolites that correlate with early-stage cancer.

Challenges and Future Directions

While many biomarkers have been identified, translating them into **clinical practice** faces challenges:

1. **Sensitivity and Specificity Issues** Many biomarkers overlap with non-cancerous conditions.
2. **Validation in Large Populations** New biomarkers must be tested across diverse populations.
3. **Standardization of Detection Methods** Consistent, reproducible techniques are needed.

Future Research Focus:

- Developing **multi-biomarker panels** combining **DNA, RNA, and proteins** for more accurate early detection.
- Integrating **AI-based analysis** to improve predictive accuracy.
- Exploring **liquid biopsy** for routine cancer screening.

3) Role of the Tumor Microenvironment (TME) in Cancer Progression

The **tumor microenvironment (TME)** refers to the complex ecosystem surrounding a tumor, including **cancer cells, stromal cells, immune cells, blood vessels, extracellular matrix (ECM), and signaling molecules**. The TME plays a crucial role in **cancer initiation, progression, metastasis, immune evasion, and therapy resistance**.

Understanding the TME is essential for developing targeted therapies that can disrupt tumor-supporting conditions and improve cancer treatment outcomes.

Key Components of the Tumor Microenvironment

1. Cancer-Associated Fibroblasts (CAFs)

CAFs are activated fibroblasts within the TME that promote tumor growth and metastasis.

Functions of CAFs in Cancer Progression:

- **Secrete Growth Factors:**
  - **Transforming Growth Factor-β (TGF-β)** and **Fibroblast Growth Factor (FGF)** stimulate cancer cell proliferation and invasion.
- **Modify ECM:**
  - Secrete **collagen, fibronectin, and hyaluronan**, which stiffen the ECM, creating a pro-tumor environment.
- **Promote Angiogenesis:**
  - Release **Vascular Endothelial Growth Factor (VEGF)** to enhance blood supply to the tumor.
- **Suppress the Immune System:**
  - CAFs release **TGF-β and IL-6**, which inhibit T-cell activity, allowing tumors to escape immune detection.

2. Immune Cells in the TME

The immune system plays a paradoxical role in cancer, either fighting or supporting tumor progression.

Key Immune Cell Types:

- **Tumor-Associated Macrophages (TAMs)**:
  - **M2-TAMs** promote tumor growth, angiogenesis, and immune evasion by secreting **IL-10, TGF-β, and VEGF**.
  - **M1-TAMs** have anti-tumor properties but are often suppressed in the TME.
- **Myeloid-Derived Suppressor Cells (MDSCs):**
  - Suppress T-cell activity and promote immune tolerance in tumors.
- **Regulatory T Cells (Tregs):**
  - Inhibit cytotoxic T-cell function, allowing tumors to evade immune destruction.

- **Cytotoxic T Cells (CTLs):**
  - Normally attack tumor cells, but their function is often suppressed by **PD-L1 expression on tumor cells**.

3. Tumor Vasculature and Angiogenesis

Tumors need a blood supply to grow beyond a few millimeters. However, tumor-induced blood vessels are **abnormal, leaky, and poorly organized**, leading to hypoxia.

Key Factors in Tumor Angiogenesis:

- **VEGF (Vascular Endothelial Growth Factor):**
  - Stimulates new blood vessel formation but leads to chaotic, dysfunctional vasculature.
- **Hypoxia-Inducible Factor-1α (HIF-1α):**
  - Drives angiogenesis in response to low oxygen levels in tumors.
- **Angiopoietins (ANG1, ANG2):**
  - Regulate blood vessel maturation and stability.

Impact on Cancer Progression:

- Hypoxia leads to **increased metastasis, therapy resistance, and immune suppression**.
- Abnormal blood flow results in **poor drug delivery**, reducing chemotherapy effectiveness.

4. Extracellular Matrix (ECM) Remodeling

The ECM provides structural support but is often **remodeled in tumors** to promote invasion and metastasis.

Key ECM Modifiers:

- **Matrix Metalloproteinases (MMPs):**
  - MMP-2 and MMP-9 degrade ECM proteins, allowing cancer cells to invade surrounding tissues.
- **Collagen and Fibronectin Deposition:**
  - Excessive ECM production stiffens the tumor, facilitating tumor growth and immune suppression.
- **Integrins and Focal Adhesion Kinase (FAK):**
  - Help tumor cells migrate and survive in new environments.

5. Cancer-Associated Signaling Pathways

The TME activates various signaling pathways that promote cancer progression.

Key Pathways:

- **TGF-β Signaling:**
  - Promotes EMT (epithelial-mesenchymal transition), leading to metastasis.
- **NF-κB Pathway:**
  - Involved in inflammation and tumor-promoting immune suppression.
- **PI3K/AKT/mTOR Pathway:**
  - Enhances tumor growth, survival, and therapy resistance.
- **Wnt/β-Catenin Signaling:**
  - Contributes to stemness and immune evasion.

How the TME Promotes Metastasis

The TME plays a crucial role in all stages of metastasis:

1. **Local Invasion:**
   - CAFs and TAMs degrade ECM and promote cancer cell motility.
2. **Intravasation:**
   - Tumor-associated blood vessels allow cancer cells to enter circulation.
3. **Circulatory Survival:**
   - Platelets shield circulating tumor cells (CTCs) from immune detection.
4. **Extravasation:**
   - Adhesion molecules (selectins, integrins) help cancer cells exit the bloodstream.
5. **Colonization and Secondary Tumor Formation:**
   - Metastatic niches provide supportive factors for tumor cell survival and proliferation.

Role of the TME in Therapy Resistance

The TME contributes to **chemotherapy, radiotherapy, and immunotherapy resistance**.

1. Chemotherapy Resistance:

- **Hypoxia-Induced Resistance:**
  - Low oxygen levels increase drug resistance by activating survival pathways.
- **Efflux Pumps:**
  - Tumor cells upregulate **P-glycoprotein (P-gp)**, which pumps chemotherapy drugs out of the cells.
- **CAFs as Drug Barriers:**
  - Dense ECM created by CAFs reduces drug penetration.

2. Immunotherapy Resistance:

- **Immune Checkpoint Activation:**
  - Tumor cells upregulate **PD-L1**, suppressing T-cell function.
- **Treg and MDSC Suppression:**

    - These immune cells block anti-tumor immunity.

3. Radiotherapy Resistance:

- **DNA Repair Mechanisms:**
    - Tumor cells in the TME have enhanced DNA repair capabilities, reducing radiation-induced cell death.
- **Hypoxia-Induced Resistance:**
    - Low oxygen levels make radiotherapy less effective.

Therapeutic Targeting of the TME

Several therapies are designed to **alter the TME** and enhance cancer treatment:

1. Anti-Angiogenic Therapy

- **Bevacizumab (Anti-VEGF Therapy)** → Normalizes blood vessels, improving drug delivery.

2. Immune Checkpoint Inhibitors

- **Anti-PD-1/PD-L1 (Nivolumab, Pembrolizumab)** → Reactivates T-cells.
- **Anti-CTLA-4 (Ipilimumab)** → Boosts immune response against tumors.

3. CAF-Targeting Therapy

- **FGF and TGF-β inhibitors** to reduce CAF-induced tumor progression.

4. ECM Modifiers

- **MMP inhibitors (Marimastat, Batimastat)** to prevent ECM degradation.

5. TME-Targeted Drug Delivery

- **Nanoparticle-based therapies** that penetrate the tumor stroma effectively.

4) Mechanisms of Resistance to Targeted Cancer Therapies

Targeted cancer therapies are designed to block specific molecular pathways involved in cancer growth and progression. Unlike traditional chemotherapy, these therapies **target oncogenic proteins, signaling pathways, or the tumor microenvironment** with greater precision, leading to fewer side effects. However, many cancers develop **resistance**, rendering these treatments ineffective over time.

Resistance to targeted therapies occurs through **genetic mutations, pathway reactivation, tumor microenvironment alterations, and adaptive cellular mechanisms**. Understanding these mechanisms can help develop **more durable treatments and combination therapies**.

Types of Resistance to Targeted Therapies

Cancer resistance to targeted therapies is classified into two major types:

1. **Primary (Intrinsic) Resistance** → Cancer cells are naturally resistant before treatment begins.
2. **Acquired Resistance** → Cancer initially responds but later develops resistance due to genetic or adaptive changes.

Key Mechanisms of Resistance

1. Genetic Mutations in Targeted Proteins

Mutations in drug targets can prevent targeted therapies from binding effectively, leading to resistance.

Examples:

- **EGFR Mutations in Lung Cancer**
  - Drugs: **Erlotinib, Gefitinib (EGFR inhibitors)**
  - Resistance: **T790M mutation in EGFR** prevents drug binding.
  - Solution: **Osimertinib (3rd-gen EGFR inhibitor)** targets T790M.
- **BCR-ABL Mutations in Chronic Myeloid Leukemia (CML)**
  - Drug: **Imatinib (Gleevec)**
  - Resistance: **T315I mutation in BCR-ABL kinase** prevents Imatinib binding.
  - Solution: **Ponatinib**, designed to inhibit T315I-mutated BCR-ABL.
- **BRAF Mutations in Melanoma**
  - Drug: **Vemurafenib (BRAF inhibitor)**
  - Resistance: **Secondary NRAS mutations or MEK reactivation**.
  - Solution: Combination with **MEK inhibitors (Trametinib)**.
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2. Activation of Alternative Signaling Pathways

Cancer cells can bypass blocked pathways by activating alternative survival pathways.

Examples:

- **PI3K/AKT/mTOR Activation**
  - Targeted therapy: **HER2 inhibitors (Trastuzumab in breast cancer)**
  - Resistance: **PIK3CA mutations or PTEN loss** activate the PI3K/AKT pathway.
  - Solution: **PI3K inhibitors (Alpelisib) + HER2 inhibitors**.
- **c-MET Amplification in Lung Cancer**
  - Targeted therapy: **EGFR inhibitors**
  - Resistance: **c-MET overexpression activates parallel survival pathways**.
  - Solution: **MET inhibitors (Capmatinib) combined with EGFR inhibitors**.

3. Tumor Microenvironment (TME)-Mediated Resistance

The TME plays a key role in drug resistance by **protecting tumor cells from therapy**.

Mechanisms:

- **Hypoxia-Induced Resistance** → Low oxygen levels increase **HIF-1α**, leading to therapy resistance.
- **Cancer-Associated Fibroblasts (CAFs)** → Secrete growth factors (TGF-β, FGF) that **reactivate tumor growth**.
- **Immune Suppression** → Tumors recruit **regulatory T cells (Tregs) and Myeloid-Derived Suppressor Cells (MDSCs)** to evade immune attack.

Solution:

- **TME-targeting therapies**, such as anti-VEGF drugs (Bevacizumab) to normalize tumor vasculature and **immune checkpoint inhibitors (Anti-PD-1/PD-L1)**.

4. Epigenetic Modifications

Epigenetic changes, such as **DNA methylation, histone modifications, and non-coding RNA regulation**, can alter gene expression and contribute to resistance.

Examples:

- **BRCA1 Methylation and PARP Inhibitor Resistance**
  - Drug: **Olaparib (PARP inhibitor for BRCA-mutant ovarian cancer)**
  - Resistance: **Loss of BRCA1 methylation restores DNA repair**.
  - Solution: Combination therapy with **epigenetic drugs (DNMT inhibitors)**.
- **EZH2 Overexpression in Lymphomas**
  - Enhances cancer stem cell survival and therapy resistance.
  - Solution: **EZH2 inhibitors (Tazemetostat) + targeted therapy**.

5. Drug Efflux and Reduced Drug Uptake

Cancer cells can develop resistance by **expelling drugs from the cell** using efflux pumps or reducing drug uptake.

Examples:

- **P-Glycoprotein (P-gp) and Multidrug Resistance (MDR1) Pumps**
  - Overexpression leads to drug efflux, reducing effectiveness.
  - Affects drugs like **TKIs, chemotherapy, and PARP inhibitors**.
  - Solution: **P-gp inhibitors + targeted therapy** (but clinical success is limited).
- **Reduced Expression of Drug Transporters**
  - Example: **Loss of OCT1 transporter in CML** leads to poor **Imatinib uptake**.

6. Cancer Stem Cell (CSC)-Mediated Resistance

Cancer stem cells (CSCs) are a subpopulation within tumors that are highly resistant to therapy.

Properties of CSCs:

- **Quiescence (Dormancy)** → Escape cell-cycle-targeted drugs.
- **High ABC Transporter Expression** → Expel drugs efficiently.
- **Self-Renewal** → Can regenerate tumors after treatment.

Solution:

- Targeting CSC pathways (Wnt, Notch, Hedgehog) using **combination therapies**.

7. Immune Evasion and Resistance to Immunotherapy

Cancer cells develop resistance to **immune checkpoint inhibitors (ICIs)** by modifying the immune response.

Mechanisms:

- **PD-L1 Upregulation** → Suppresses T-cell function.
- **Loss of Antigen Presentation** → Tumor cells downregulate **MHC-I**, making them "invisible" to T-cells.
- **TME Immunosuppression** → High levels of **Tregs, MDSCs, and TAMs** suppress anti-tumor immunity.

Solution:

- **Combination of ICIs (PD-1 + CTLA-4 inhibitors)**.
- **Cytokine therapy (IL-2, IFN-γ) to boost T-cell activity**.

5) Cancer Stem Cell (CSC) Biology and Therapeutic Targeting

Cancer stem cells (CSCs) are a **small subpopulation of cancer cells** with **self-renewal, differentiation, and therapy resistance capabilities**. They contribute to **tumor initiation, progression, metastasis, and recurrence**.

Understanding CSC biology is essential for developing **targeted therapies** that eliminate CSCs and prevent cancer relapse.

1. Characteristics of Cancer Stem Cells (CSCs)

CSCs share properties with normal stem cells but drive tumor growth and resistance.

Key Features:

Self-Renewal Can indefinitely produce identical CSCs.
Differentiation Can generate heterogeneous cancer cell populations.
Therapy Resistance Resistant to chemotherapy, radiotherapy, and targeted therapy.
Dormancy & Quiescence Can remain inactive and evade treatment.
Metastatic Potential High ability to invade and colonize new organs.

CSC Markers (Identification of CSCs)

CSC populations express specific **surface markers**, which vary by cancer type:

Cancer Type	CSC Markers
Breast Cancer	$CD44^+CD24^-$, $ALDH1^+$
Colorectal Cancer	$CD133^+$, $LGR5^+$
Lung Cancer	$CD133^+$, $ALDH1^+$
Glioblastoma	$CD133^+$, $Nestin^+$
Leukemia (AML, CML)	$CD34^+CD38^-$

2. Cancer Stem Cell Niches & Regulation

CSCs reside in specialized **niches** that regulate their function and therapy resistance.

Types of CSC Niches:

- **Hypoxic Niche** Low oxygen (hypoxia) activates **HIF-1α**, maintaining CSCs.
- **Vascular Niche** Blood vessels provide nutrients and support CSC survival.
- **Inflammatory Niche** Cytokines (IL-6, TNF-α) promote CSC self-renewal.

Key Signaling Pathways Regulating CSCs:

- **Wnt/β-Catenin Pathway** Controls CSC proliferation and therapy resistance.
- **Hedgehog (Hh) Pathway** Promotes CSC self-renewal in brain and pancreatic cancers.
- **Notch Pathway** Enhances CSC survival, especially in breast cancer.
- **PI3K/AKT/mTOR Pathway** Drives CSC expansion and therapy resistance.

3. CSCs and Therapy Resistance

CSCs evade conventional therapies through several mechanisms:

1. Chemotherapy Resistance

- **Drug Efflux Pumps** CSCs express **ABCG2, MDR1/P-gp**, which expel drugs.
- **Quiescence** Many CSCs remain dormant, avoiding drugs that target dividing cells.
- **Enhanced DNA Repair** High activity of **BRCA1, ATM, and ATR** repairs chemotherapy-induced DNA damage.

2. Radiotherapy Resistance

- **ROS Scavenging** CSCs have high antioxidant capacity, reducing radiation-induced DNA damage.
- **DNA Damage Repair** CSCs quickly repair radiation damage via the **NHEJ pathway**.

3. Immune Evasion

- **Low MHC-I Expression** CSCs escape T-cell recognition.
- **PD-L1 Overexpression** Inhibits immune response, preventing immune-mediated killing.

4. Therapeutic Targeting of Cancer Stem Cells

Since CSCs contribute to therapy resistance and relapse, researchers are developing **CSC-specific treatments**.

1. Targeting CSC-Specific Signaling Pathways

- **Wnt Inhibitors PRI-724, LGK974** (target β-Catenin signaling).
- **Hedgehog Inhibitors Vismodegib, Sonidegib** (block Smoothened protein).
- **Notch Inhibitors Gamma-secretase inhibitors (RO4929097, BMS-906024)**.
- **PI3K/AKT/mTOR Inhibitors Everolimus, Alpelisib** (block survival pathways).

2. Immunotherapy for CSCs

- **Immune Checkpoint Inhibitors (ICIs) Anti-PD-1 (Pembrolizumab, Nivolumab)** to restore immune response.
- **CAR-T Therapy** Engineered T-cells targeting **CD133, EpCAM** on CSCs.

3. Epigenetic Targeting of CSCs

- **DNMT Inhibitors (Decitabine, Azacitidine)** Reprogram CSCs to be more sensitive to therapy.
- **HDAC Inhibitors (Vorinostat, Panobinostat)** Disrupt CSC survival pathways.

4. Targeting CSC Metabolism

CSCs rely on unique metabolic pathways for survival:

- **Metformin (Anti-Diabetic Drug)** Inhibits CSC mitochondrial metabolism.
- **Glutaminase Inhibitors (CB-839)** Block CSC energy production.

5. Nanoparticle-Based Drug Delivery

Nanotechnology enhances drug penetration into CSC niches:

- **Lipid Nanoparticles (LNPs)** Deliver siRNA to silence CSC-specific genes.
- **Gold Nanoparticles** Selectively target and kill CSCs with **photothermal therapy**.