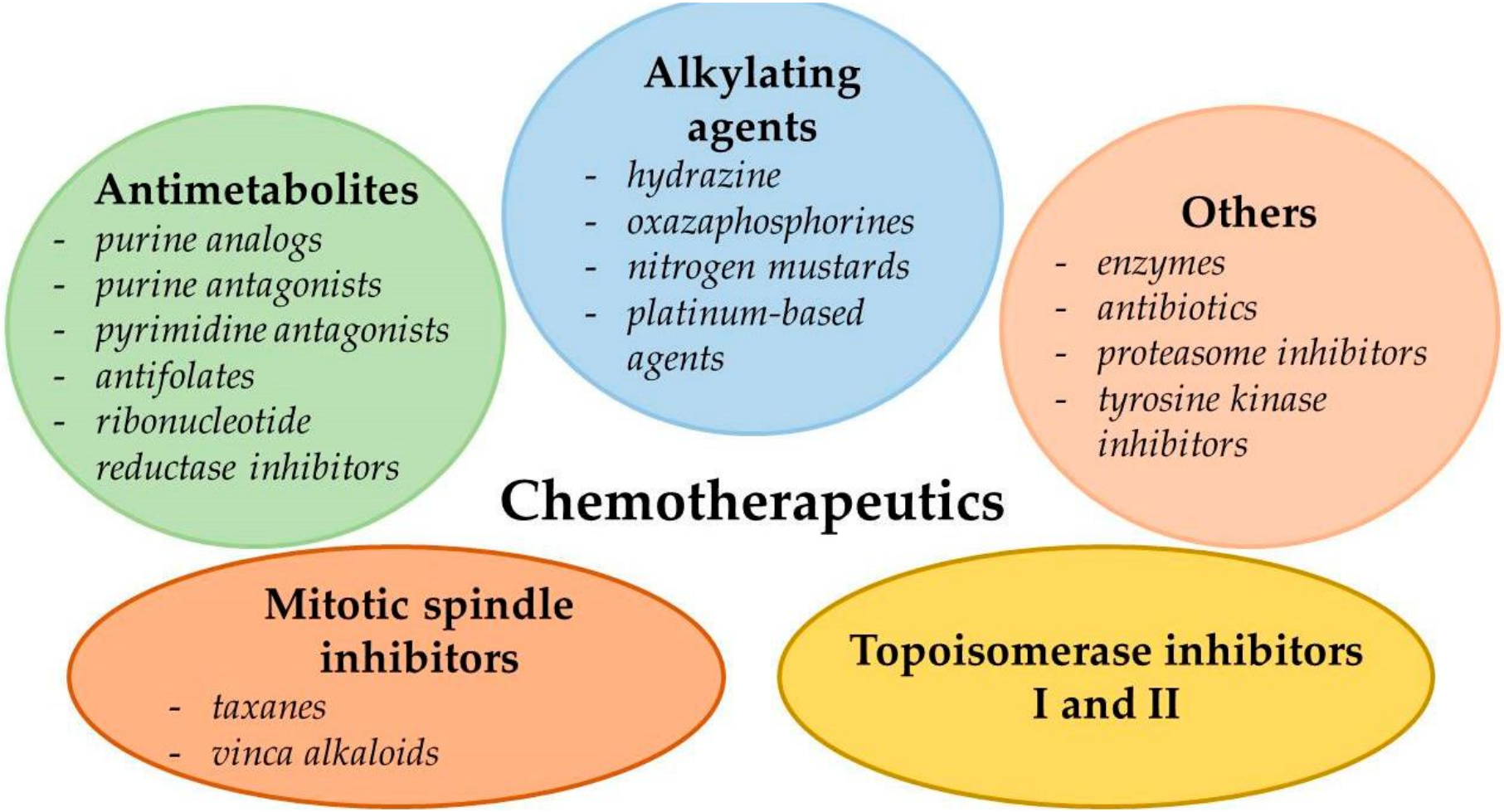
# Cancer Drug Resistance

# Chemotherapy

- Single-agent chemotherapy
- Poly chemotherapy
- Short interval-high dose chemotherapy
- High doses of chemotherapy with growth factors
- Targeted therapy
- Immunotherapy

# Common chemotherapeutic drugs



# Tumor burden and growth kinetics

- log-kill hypothesis **proposes that the magnitude of tumor cell kill by anticancer drugs is a logarithmic function**
  - combining multiple drugs that individually kill a logarithmic fraction of cells over multiple cycles would permit sequential decreases in tumour burden until the disease was fully eradicated
- **Goldie–Coldman** hypothesis
  - The probability that a cancer contains drug-resistant clones depends on the mutation rate and the size of the tumour
  - alternating non-cross-resistant combinations of chemotherapy, rather than administering all therapies at once (which is often limited by toxicity), is superior in preventing drug resistance as compared to sequential therapies

# Tumor burden and growth kinetics

- tumours with low rates of growth -long survival but incurable with cytotoxic chemotherapy or even with targeted therapies
- tumours that grow at higher speeds can be exquisitely sensitive to chemotherapy
- **Norton–Simon hypothesis**
  - tumours grow in a sigmoidal manner— exponentially faster at low tumour burdens and subsequently approaching a plateau with slower growth rates as they reach a larger size
  - When the drug reduces the tumor size-tumor cells resume exponential growth

# Tumor heterogeneity

- relatively slow rate of age-related mutations to bursts of dramatic and catastrophic events that are induced by genomic instability, chromothripsis and chromosomal instability
- Mutations evolve - exogenous exposures, internal environmental dynamics and cancer therapies themselves
- in low-grade gliomas, chemotherapy with temozolomide can result in hypermutated tumours at recurrence - the transformation of tumours to highly aggressive glioblastoma multiforme
- Catalog of clonal mutations –rather than sequencing single tumor

# Physical barriers

- pro-tumorigenic hypoxic environment and decreasing the effective exposure of a tumour to drugs
- combination of anti-angiogenic tyrosine kinase inhibitors and anti-PD-1/PD-L1 antibodies has also demonstrated what appears to be synergistic activity
- sanctuary sites', - central nervous system (CNS) and the physical boundary imposed by the blood–brain barrier

# Immune system and tumour microenvironment

- preventing immune clearance of tumour cells, hindering drug absorption and stimulating paracrine growth factors to signal cancer cell growth
- ‘immune deserts’—made of regulatory T cells, myeloid-derived suppressor cells, tumour-associated macrophages, cytokines - major impediment to checkpoint inhibitors
- immunotherapy-resistant tumours have a low mutational burden, which leads to a paucity of neoantigens

# Undruggable genomic drivers

- *MYC*, *RAS* and *TP53*
- Target indifference—in which the effects of targeting an oncogenic driver are attenuated by downstream or parallel alterations in the pathway—can drive resistance

# Selective therapeutic pressure

- Conventional chemotherapy and radiotherapy enhance genomic instability, with massive and widespread effects on surviving cells and non-cancer cells
- Adaptive response during the chemotherapy
- Non-genetic relief of negative feedback mechanisms

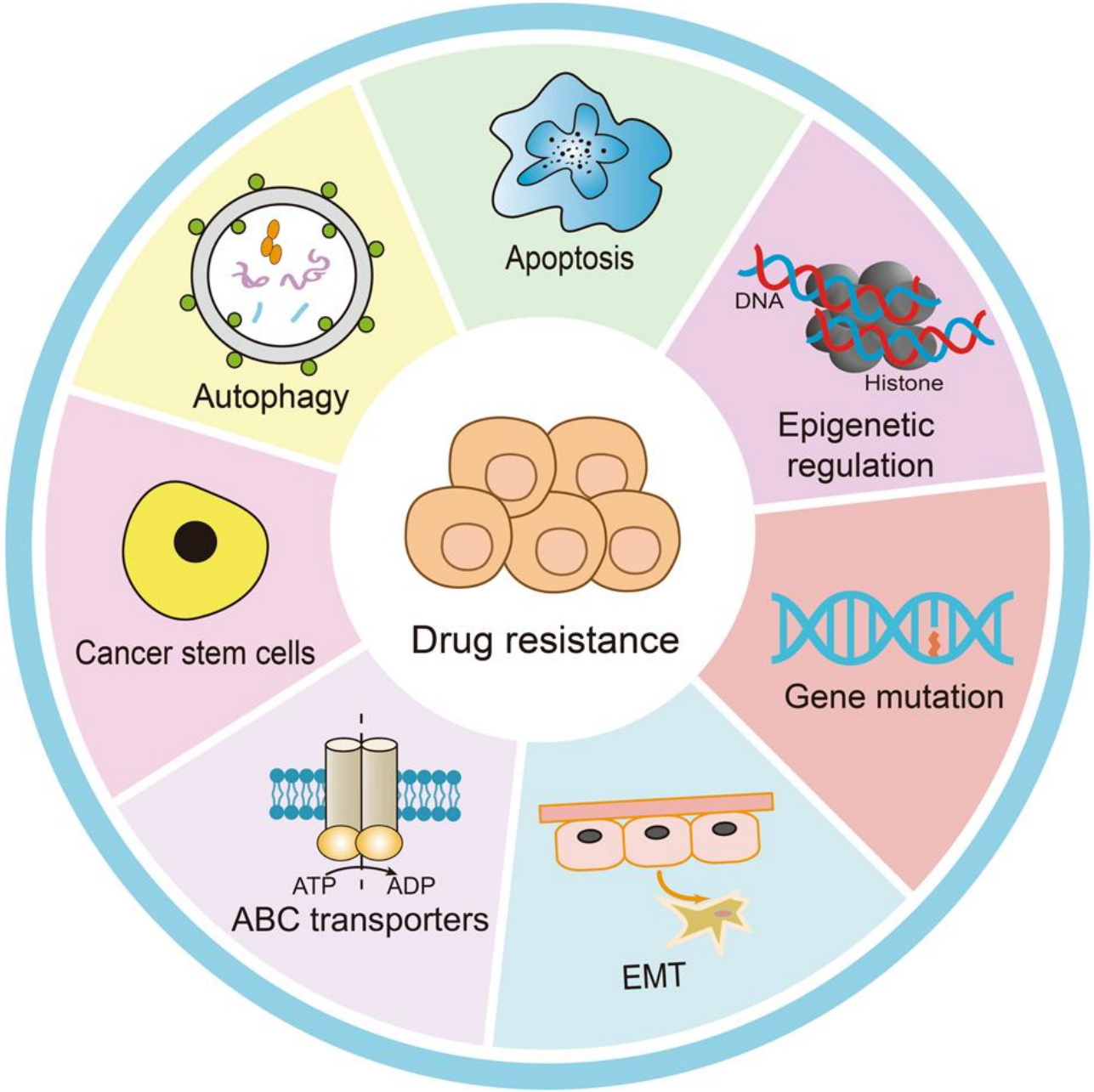
# Introduction

Cancer drug resistance refers to the failure of cancer cells to respond to treatment.

This can occur either from the outset (intrinsic) or develop over time (acquired).

Resistance arises from a complex interplay between cancer cells, therapies, and the patient's body, including the immune system and tumor microenvironment.

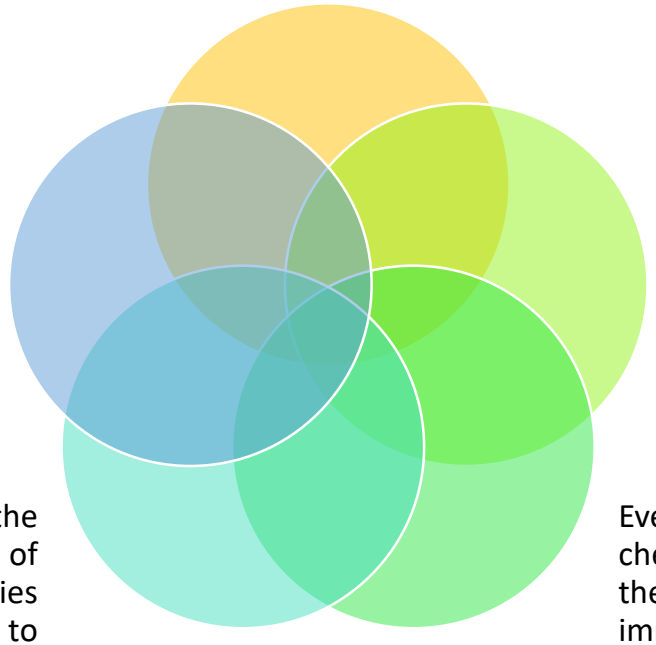
Like antibiotic resistance in infections, cancer drug resistance remains one of the biggest challenges in oncology.



# Why Addressing Cancer Drug Resistance Matters?

Cancer drug resistance is a major barrier to achieving sustained remission in cancer patients.

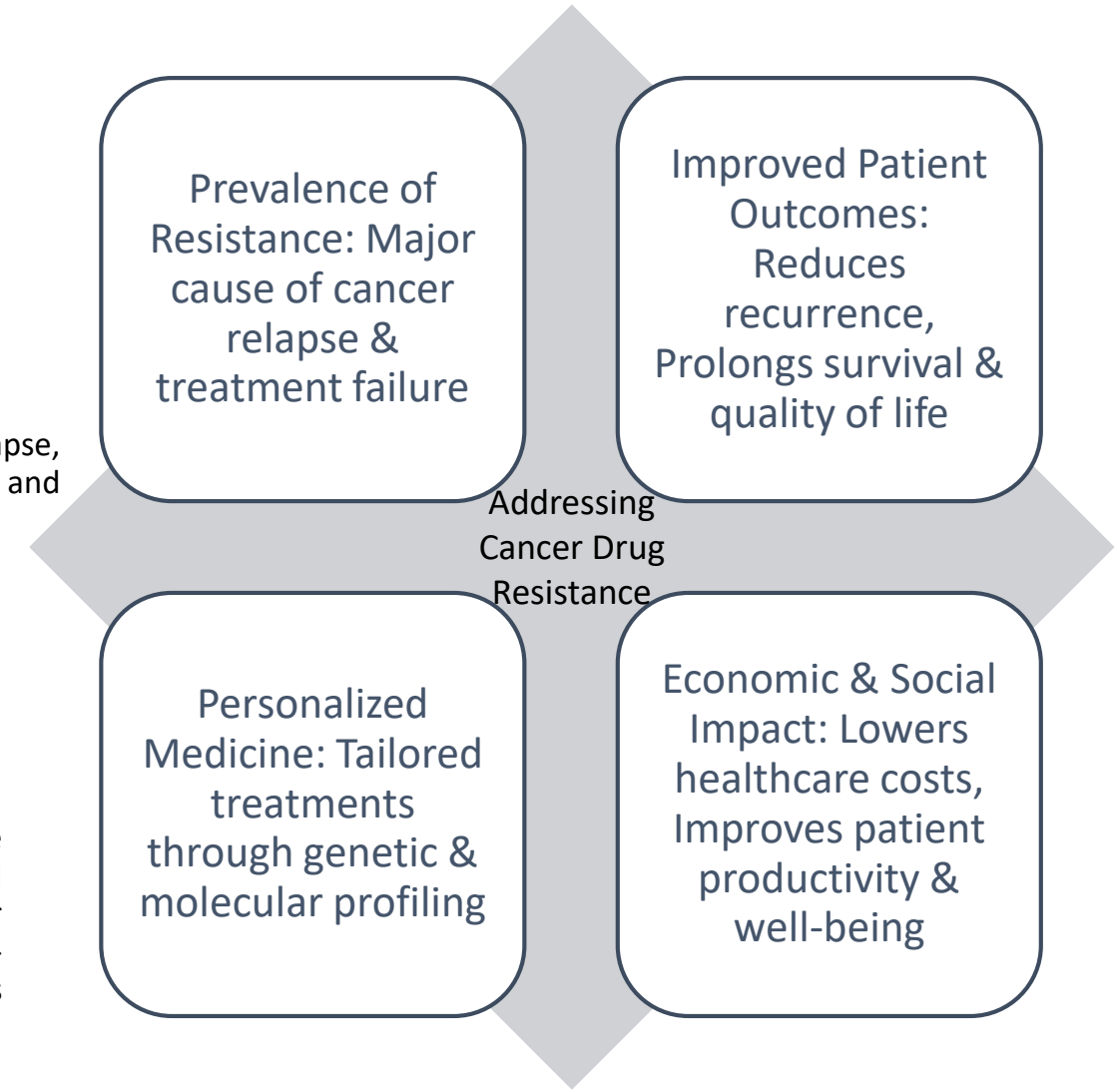
Earlier detection, stronger anti-tumor response, adaptive monitoring, and identifying new therapeutic vulnerabilities need to be prioritized.



It leads to tumor relapse, disease progression, and death.

This necessitates the development of combination therapies and novel approaches to improve survival outcomes.

Even with initially effective chemotherapy, targeted therapy, or immunotherapy, long-term treatment success is reduced.



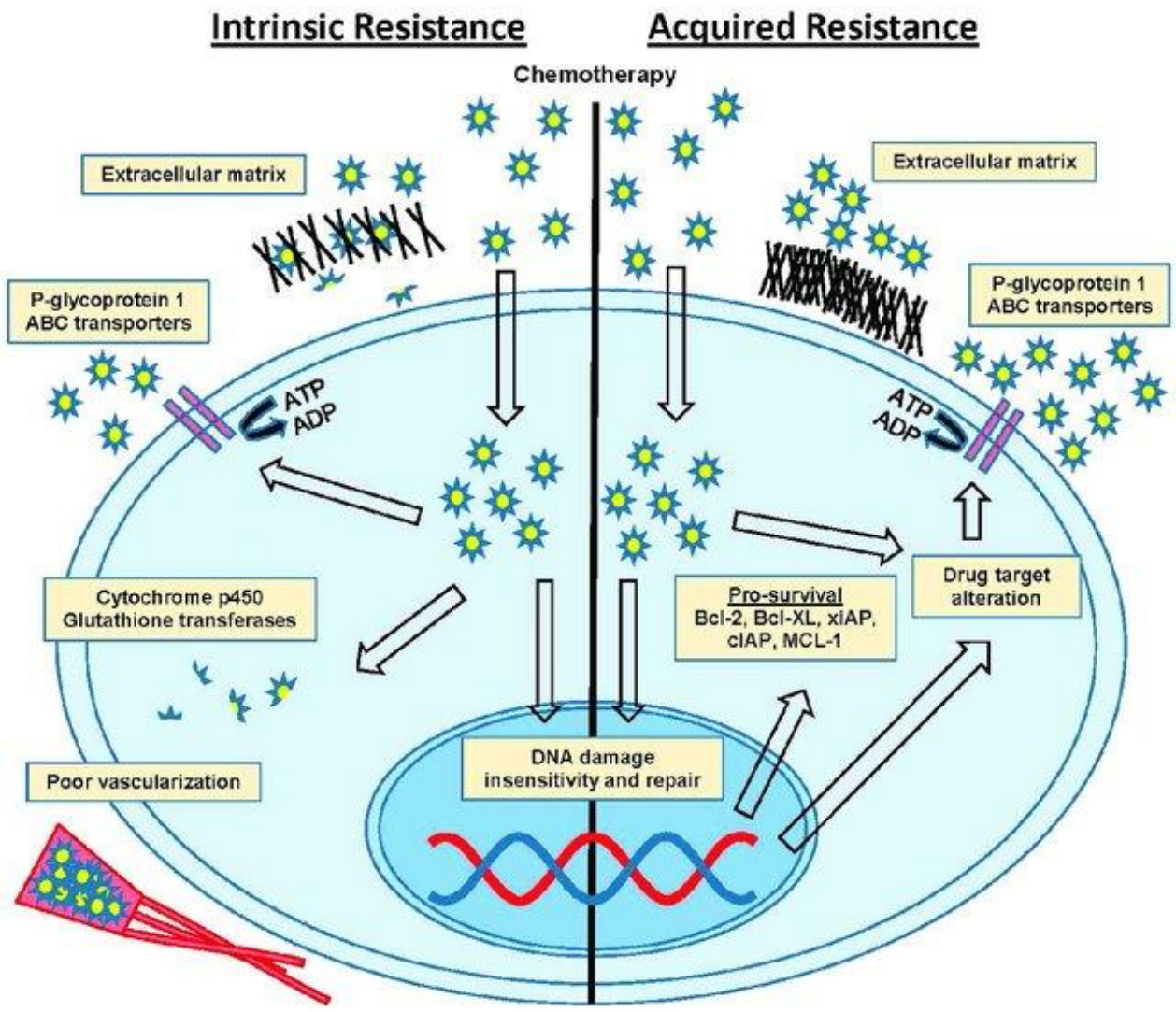
# Types of Cancer Drug Resistance

## Intrinsic Resistance

| | | |
|---|---|---|
| Tumor cells pump out drugs using ABC transporters, reducing drug concentration. | Detoxifying enzymes like cytochrome P450 and glutathione transferases break down drugs. | Poor blood supply to the tumor decreases drug delivery. ECM interactions and secreted factors create a protective microenvironment. |

## Acquired Resistance

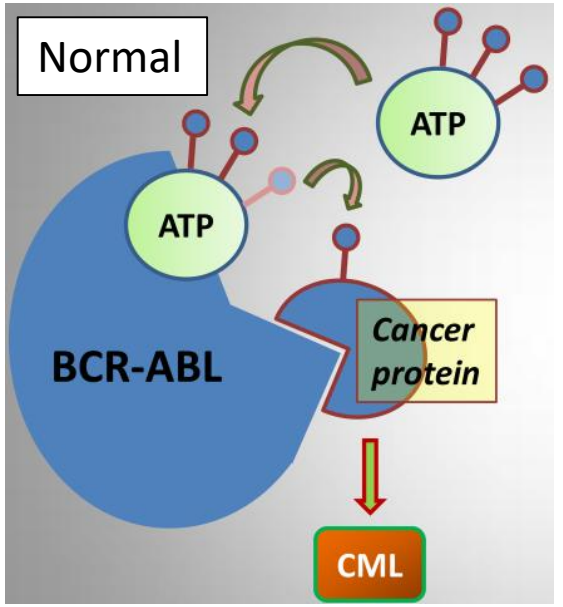
| | | | |
|---|---|---|---|
| Tumor cells increase anti-apoptotic proteins (BCL-2, BCL-XL, MCL-1, cIAP). | DNA repair mechanisms become more efficient, tolerating damage. | ECM and surface proteins change to support survival. | Drug targets mutate or are bypassed through alternate pathways. |

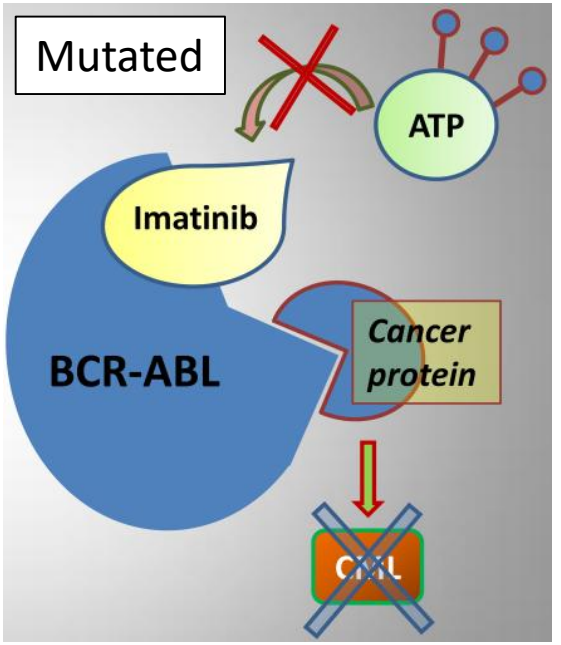


# Types of Drug Resistance in Cancer

## 1. Single Drug Resistance (SDR):

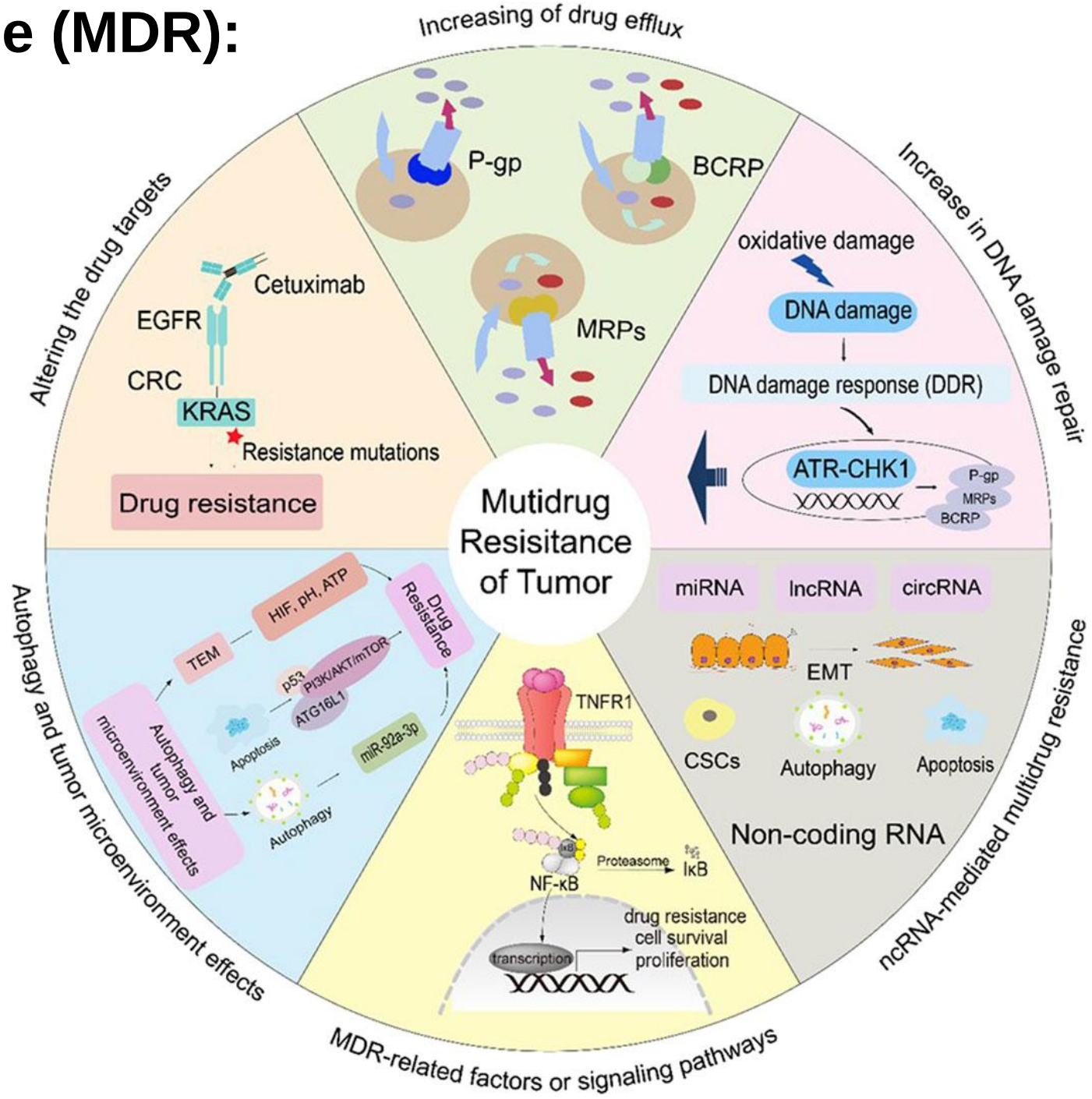
- SDR occurs when cancer cells become resistant to one specific drug.
- Common causes:
  - Mutations in the drug's target protein.
  - Enhanced drug metabolism or inactivation.
  - Changes in cell survival pathways.
- Example: Mutations in the ATP-binding site of BCR-ABL reduce imatinib binding, leading to resistance against imatinib in chronic myeloid leukemia (CML).
- It often leads to treatment failure and the need for alternative drugs or combination approaches.



# 2. Multiple Drug Resistance (MDR):

In Multidrug resistance (MDR), cancer cells become resistant to a broad range of structurally and functionally unrelated anticancer drugs.



# Factors contributing to MDR in tumor cells:

| | |
|---|---|
| **Increased Drug Efflux:** | Overexpression of ABC transporters — ABCB1 (P-gp), ABCC1 (MRP), and ABCG2 (BCRP) — reduces intracellular drug levels by actively pumping drugs out of cells. |
| | Long-term chemotherapy induces their expression. |
| **Altered Drug Targets:** | Target mutations reduce drug binding and efficacy. |
| | For example, S492R mutation in EGFR leads to cetuximab resistance in colorectal cancer. |
| **Enhanced DNA Damage Repair:** | Cancer cells rely on hyperactive DNA damage response (DDR) and checkpoints to survive therapy-induced stress. |
| | Loss of G1 checkpoint increases dependence on the ATR-CHK1 pathway. ATR-CHK1 activation supports DNA repair and reduces drug cytotoxicity. |

# Factors contributing to MDR in tumor cells:

## MDR-Related Factors and Signaling Pathways:

- Key pathways such as PI3K/AKT, NF-κB, and MAPK promote drug resistance.
- These pathways upregulate ABC transporters and anti-apoptotic factors.
- Crosstalk between signaling pathways enhances tumor adaptability to chemotherapy.

## ncRNA-Mediated MDR:

- MicroRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs) regulate MDR-related genes.
- ncRNAs can modulate expression of ABC transporters, apoptosis-related genes, and cell survival pathways.
- Dysregulation of ncRNAs contributes to acquired resistance.

## Autophagy and Tumor Microenvironment (TME) Effects:

- Autophagy can promote cancer cell survival under drug-induced stress by recycling cellular components.
- TME factors such as hypoxia, stromal cells, and extracellular vesicles influence MDR by altering drug metabolism and delivery.
- TME also induces signaling pathways that sustain drug resistance and tumor progression.

# 3. Targeted Therapy Resistance:

Despite the success of targeted therapies in cancer treatment, resistance inevitably emerges through multiple mechanisms that allow cancer cells to evade therapeutic pressure.

Mechanisms of resistance against targeted therapies for cancer include:

**On-target resistance through target reactivation**

Cancer cells can restore the function of the drug target through secondary mutations (e.g., EGFR T790M), gene amplifications, or alternative splicing, which contributes to drug resista

**Activation of upstream or downstream signaling pathways**

When the direct drug target is blocked, cancer cells may activate other nodes in the same pathway (such as RAS or NF1 mutations in melanoma) or downstream effectors to sustain growth and survival.

**Engagement of parallel oncogenic pathways**

Cancer cells often activate alternate pathways (such as MET or HER2 amplification in EGFR-mutant cancers), which can bypass the inhibited target and maintain oncogenic signaling.

**Phenotypic changes and lineage plasticity**

Cancer cells can undergo transitions like epithelial-to-mesenchymal transition (EMT) or histological changes, leading to loss of dependence on the original target.

**Adaptive survival programs and transcriptional changes**

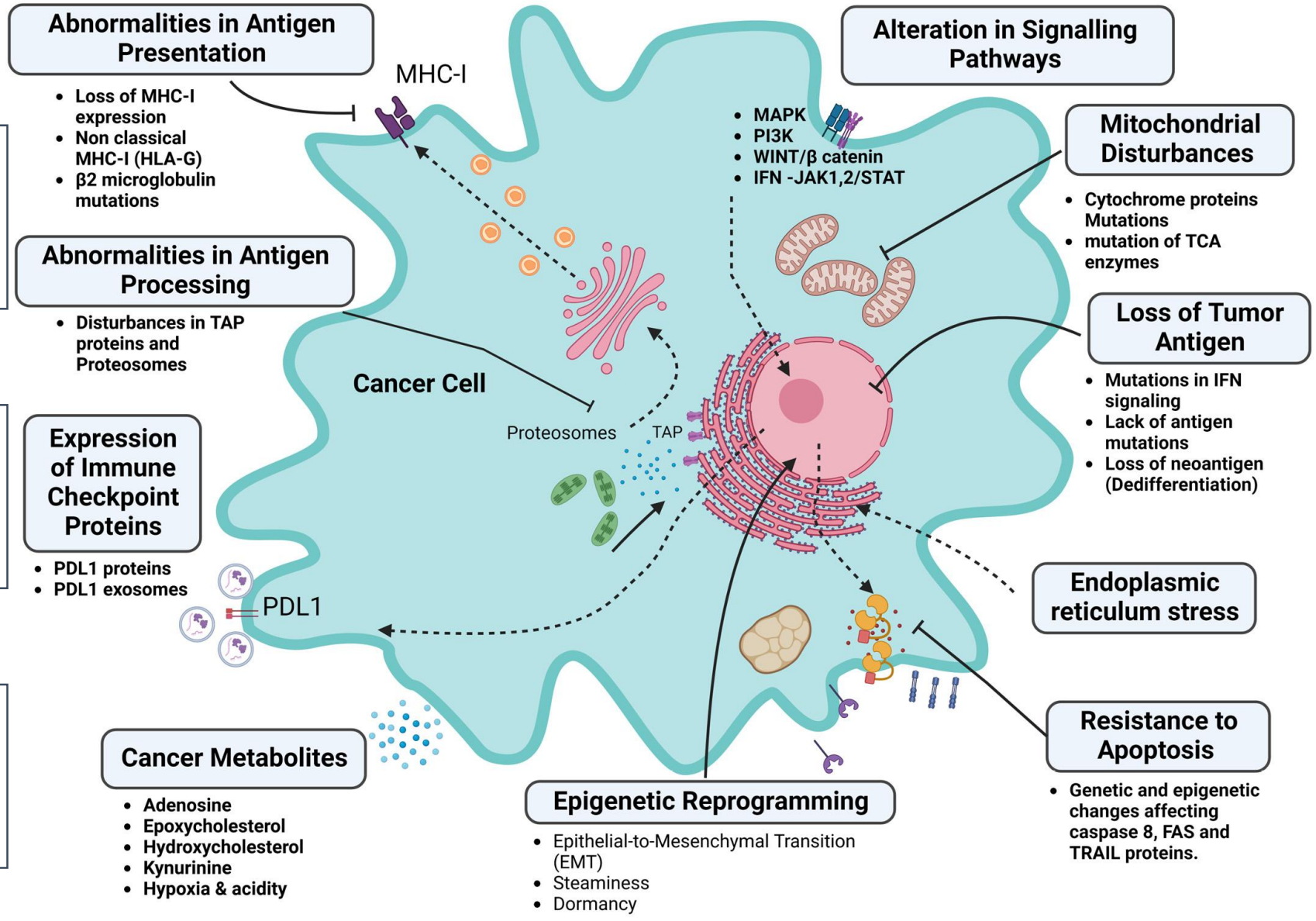
Rapid, non-genetic adaptations — such as activation of NF-κB or YAP1 signaling — can help cancer cells survive initial drug exposure and contribute to long-term resistance.

# 4. Immunotherapy Resistance:

Immunotherapy has significantly advanced cancer treatment, offering hope for long-term remission.

However, many patients either do not respond or develop resistance over time.

This resistance arises from complex interactions between cancer cells and the tumor microenvironment.



# Mechanisms of Immunotherapy Resistance:

**Loss of Tumor Antigen Recognition:**

Tumors may lose or alter neoantigens, reducing immune visibility. Defective antigen processing and presentation, including MHC-I loss or β2-microglobulin mutations, impair T cell activation.

**Upregulation of Immune Checkpoint Molecules:**

Tumor cells increase PD-L1 expression to suppress T cell function. PD-L1 can also be secreted via exosomes, influencing nearby cancer cells and contributing to resistance.

**Activation of Alternative Oncogenic Pathways:**

Pathways like MAPK, PI3K, and Wnt/β-catenin reduce immune cell infiltration and support an immune-evasive environment.

# Mechanisms of Immunotherapy Resistance:

### Metabolic Modulation in the Tumor Microenvironment:

Hypoxia, lactic acid build-up, and secretion of metabolites (adenosine, kynurenine) inhibit effector T cells and promote suppressive cells.

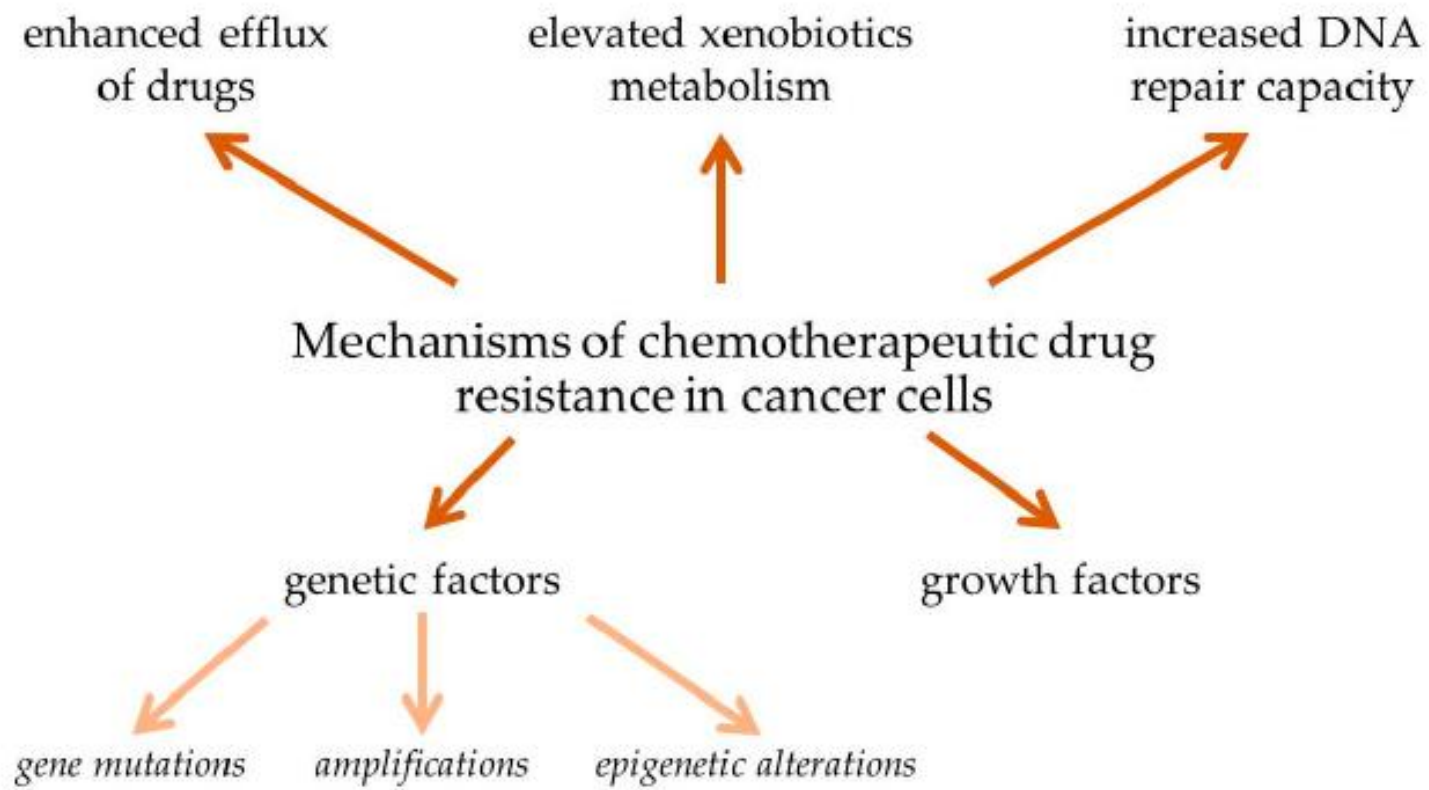
### Immunosuppressive Tumor Microenvironment:

The presence of Tregs, MDSCs, M2 macrophages, and cancer-associated fibroblasts creates an environment hostile to cytotoxic immune cells.

### Epigenetic Reprogramming and EMT:

Epigenetic alterations and epithelial-to-mesenchymal transition (EMT) reduce MHC expression and increase PD-L1, leading to immunotherapy resistance.

# Mechanisms of cancer drug resistance



# Drug absorption, distribution, metabolism and excretion (ADME)-related genes

- ADME-related genes refer to the genes involved in the absorption, distribution, metabolism, and excretion of drugs, which are composed of 266 extended genes and 32 core genes
- Based on their roles in the pharmacokinetic process, these genes can be classified into various categories such as drug transporters, modified proteins, and drug metabolic enzymes
- Increasing evidence suggests that polymorphisms in ADME-related genes play a significant role in individual differences in carcinogenesis and drug response
- In the context of gastric cancer, the decreased expression of ALDH6A1 may serve as a prognostic indicator and a determinant of response to immunotherapy
- The prognostic signature for breast cancer was comprised of four genes: ABCB5, ATP7B, KCNJ11, and TAP1.

# Drug absorption, distribution, metabolism and excretion (ADME)-related genes

- ABCB5 blocking reversed the drug resistance of Merkel Cell Carcinoma (MCC) in vivo xenotransplantation model and inhibited tumor growth, indicating that ABCB5 might be related to the chemical resistance of MCC.
- Among individuals with CRC receiving oxaliplatin chemotherapy, Elevated ATPase copper transporting beta (ATP7B) levels were linked to a poor prognosis.
- Zhang et al. found KCNJ11 formed a complex with lactate dehydrogenase A and acted as an oncogene in hepatocellular carcinoma (HCC), indicating that it may be possible to design a candidate strategy for preventing HCC that targets KCNJ11.
- Ling et al. showed that TAP1 down-regulation was both a poor predictor of outcome for patients with stage I-II CRC and a putative tumor immune escape mechanism.
- Segami et al. revealed that better overall survival was correlated with higher TAP1 expression in advanced gastric cancer

# Role of drug efflux pumps (p-gp)

The P-glycoprotein (P-gp) pathway is significant in cancer drug resistance as it functions as a drug efflux transporter, actively pumping chemotherapy drugs out of cancer cells, thereby reducing their efficacy.

Targeting P-gp can enhance the response of tumors to chemotherapy, making it a critical focus in overcoming chemoresistance.

Additionally, non-coding RNAs, such as microRNAs, can regulate P-gp levels, further influencing drug sensitivity in various cancers.

The suppression of P-glycoprotein (P-gp) activity is crucial for overcoming chemoresistance in human cancers because it directly impacts the regulation of drug resistance mechanisms.

By inhibiting P-gp, the effectiveness of chemotherapeutic agents can be enhanced, allowing for better therapeutic outcomes in cancer treatment.

This is particularly important as P-gp upregulation is associated with the development of drug resistance in various cancers.

# Role of drug efflux pumps (p-gp)

The expression of P-glycoprotein (P-gp) is negatively associated with chemotherapy response in various cancers, including leukemias.

High levels of P-gp have been linked to poor chemotherapy outcomes, as seen in studies where patients with P-gp expression had a significantly higher risk of being refractory to treatment.

For instance, in acute myeloid leukemia, P-gp levels increased from 24% at diagnosis to 67% during tumor recurrence, correlating with reduced overall survival rates.

Drug efflux transporters like P-glycoprotein (P-gp) regulate cancer chemoresistance by actively transporting drugs out of tumor cells, thereby reducing intracellular drug accumulation.

P-gp expression can be upregulated by oncogenic pathways and downregulated by tumor-suppressor factors.

Additionally, non-coding RNAs can influence P-gp activity, contributing to the development of drug resistance in cancers.

Exosomes also play a role by transferring proteins and non-coding RNAs that can modulate P-gp levels, further impacting drug resistance mechanisms.

# Role of drug efflux pumps (ABC proteins)

Human ATP-binding cassette (ABC) transporters are ubiquitously expressed and transport a broad range of endogenous and xenobiotic substrates across extra and intracellular membranes.

Mutations in ABC genes cause 21 monogenic diseases, and polymorphisms in these genes are associated with susceptibility to complex diseases.

ABC transporters also play a major role in drug bioavailability, and they mediate multidrug resistance in cancer.

At least 13 ABC transporters were shown to be involved in drug resistance in vitro.

ABC transporters comprise 44 membrane transporters and four nontransporter ABC proteins

ABC transporters translocate a wide variety of substrates, including lipids, peptides, sterols, and vitamins, across cellular membranes and have a broad range of cellular roles

ABCB1 was the first ABC transporter found to confer multidrug resistance in cancer.

ABC transporters represent an important line of defense against carcinogens. Their expression at physiological barriers and excretory organs restricts exposure to environmental carcinogens and increases their clearance.

# Role of drug efflux pumps (ABC proteins)

ABC transporters also play a role in reactive oxygen species (ROS) homeostasis.

It is accepted that a high level of ROS not only initiates DNA damage and carcinogenesis but also induces regulatory pathways, such as proliferation, angiogenesis, and metastasis.

The tumorigenesis promoting ROS level involves a delicate balance, because an excessively high concentration of ROS induces apoptosis and senescence of tumor cells.

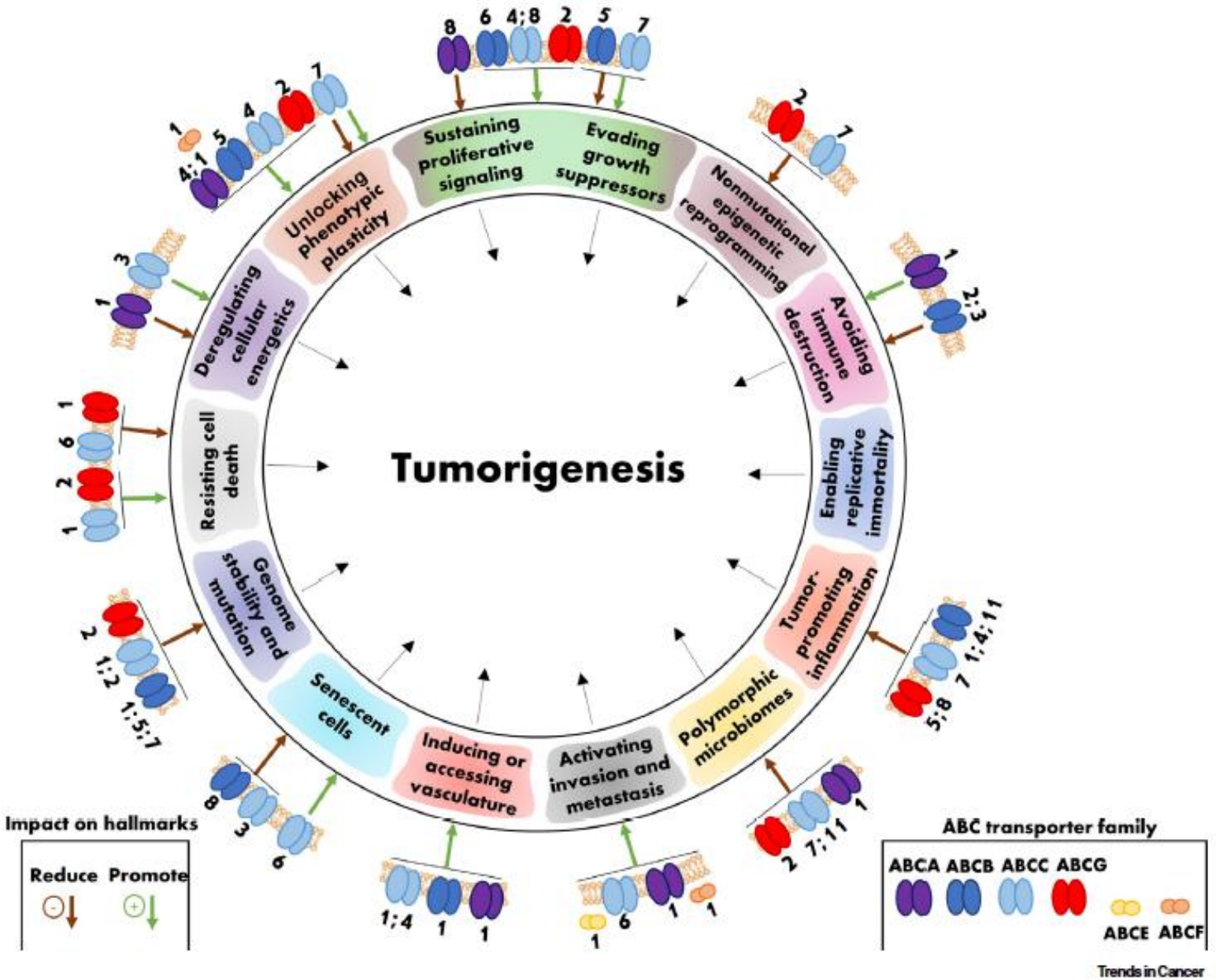
ABCG2 plays a role in the redox balance and antioxidant production by modulating the intracellular concentration of glutathione.

There are 12 members of the ABCA family, all of which play a significant role in cholesterol homeostasis and membrane lipid trafficking

# Role of drug efflux pumps

Key figure

The diverse roles of ATP-binding cassette (ABC) transporters in tumorigenesis



# Increased DNA Repair Capacity

DNA repair endonuclease XPF and DNA excision repair protein ERCC1 involved in the nucleotide excision repair (NER) pathway are essential for the efficient repair of DNA damage induced by crosslinking and platinum-based agents

A significant correlation between overexpression of both the XPF and ERCC-1 proteins and the development of cisplatin resistance in cancer cells was shown

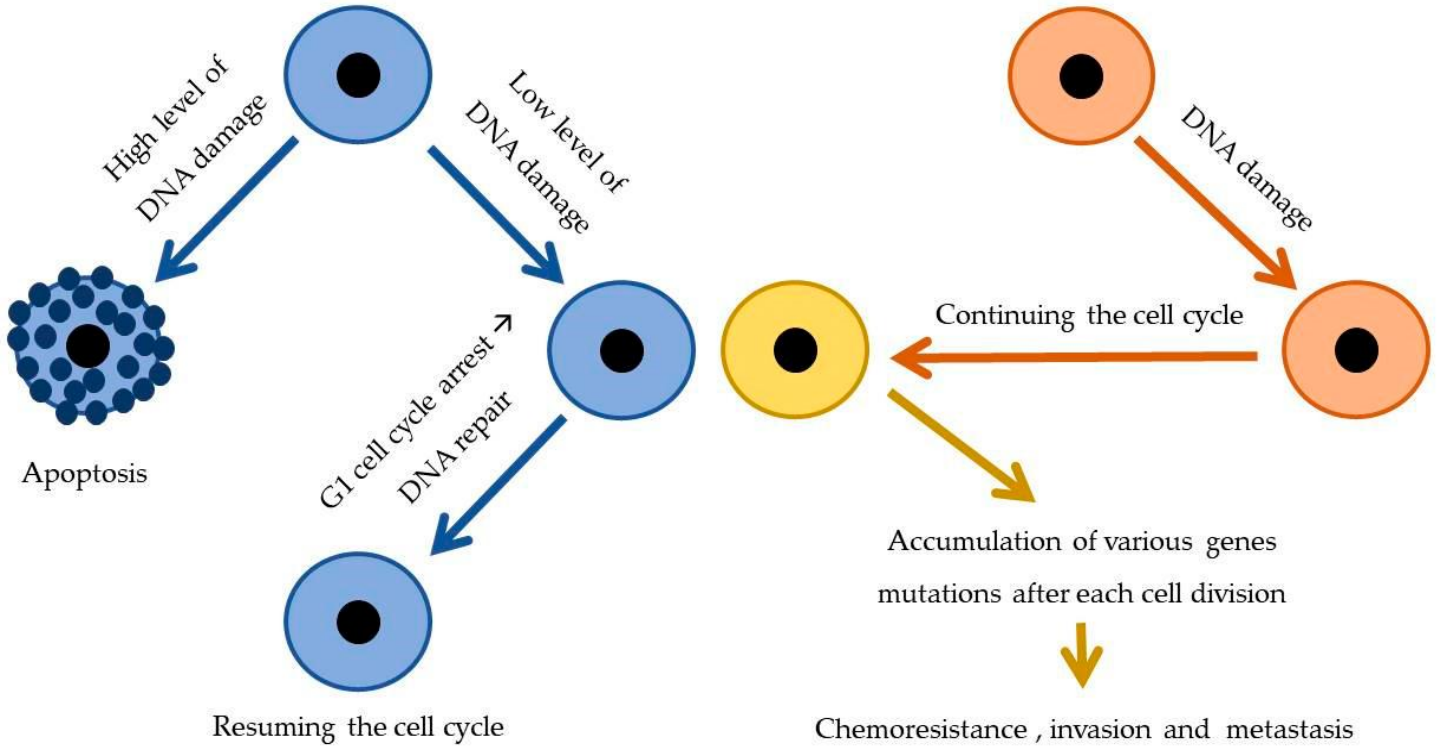
RAD51 is a protein involved in HR pathway responsible for DNA DSB repair. It binds to ssDNA and assists in HR repair by exchanging DNA strand breaks.

Enhanced HR and overexpression of RAD51 have been found in multiple myeloma cells.

Furthermore, high RAD51 expression in vivo has been shown to be correlated with chemoresistance and poor patient survival.

# Gene Mutations

- Gene mutations, which are commonly observed in tumor cells are considered one of the main causes of the failure of chemotherapy treatment.
- The best explanation of MDR development in cancer cells is their aneuploidy nature.
- Researchers have suggested that frequently losing chromosomes or their reassortments during mitosis are responsible for losing drug-sensitive genes or for changes in biochemical pathways, which both seems to be crucial in chemotherapeutic drug resistance.
- Losing the tumor-suppressive activities by missense mutations in the *TP53* gene, which are especially widespread in human cancers, reverses the protective role of the *TP53* pathway by initiating chemoresistance, invasion, and metastasis.



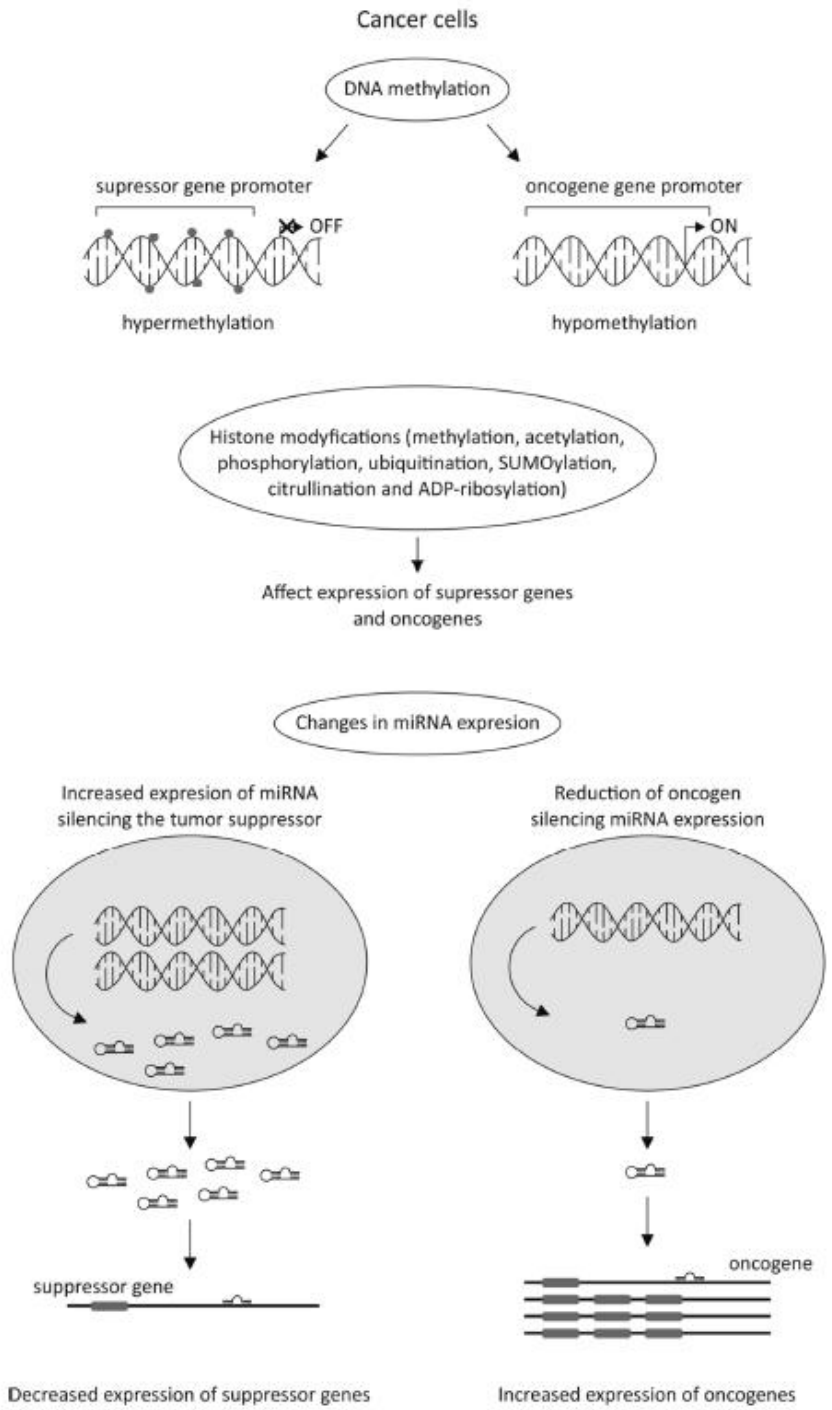

- Furthermore, function of the chimeric BCR-ABL gene seems to be key for initiation and maintenance of tumorigenicity, especially in chronic myeloid leukemia (CML). The oncogenic gene product increases frequency of cell division, blocks DNA repair, and inhibits apoptosis.

# Epigenetic Alterations

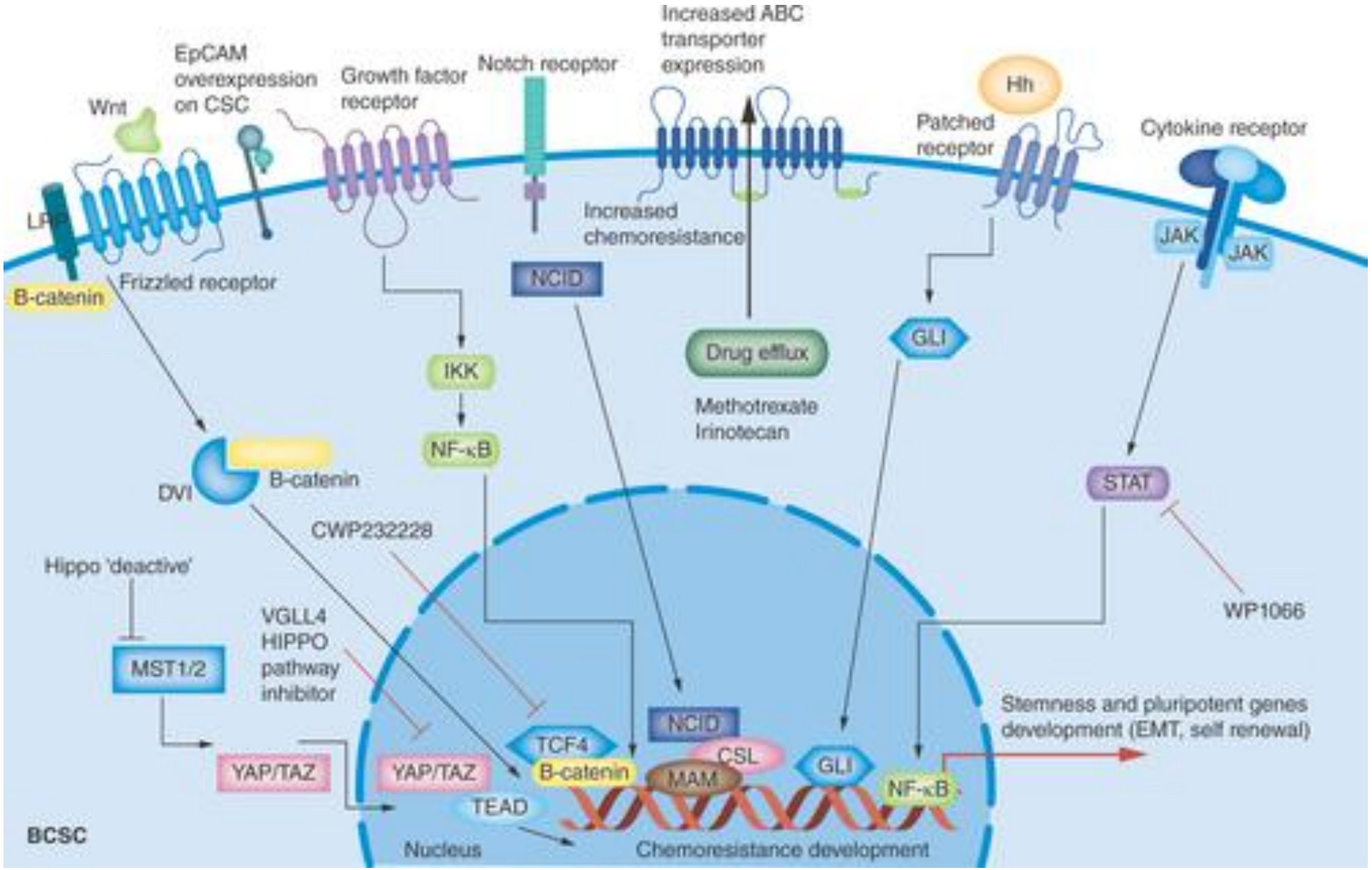
The latest data strongly emphasizes the significant role of epigenetic alterations in cancer cells in anticancer drug resistance.

Silencing tumor suppressor genes by their DNA hypermethylation or enhancing the expression of oncogenes by their DNA hypomethylation could be the potential factors involved in cancer development.

Currently, only two classes of epigenetic drugs have been approved by the FDA, i.e., DNA methylation inhibitors (iDNMT), including 5-azacitidine and 5-aza-20-deoxycytidine (decitabine; DAC), as well as histone deacetylase inhibitors (iHDACs), such as Vorinostat, Belinostat, Romidepsin, and Panobinostat



# Signaling pathways in cancer survival and resistance to drugs



# Tumor microenvironment (TME)

Low extracellular pH, increased ROS concentrations, hypoxia, and the overexpression of certain proteases and factors are TME characteristics

TME has its own blood supply, lymphatic and neurological systems, stroma, immune cells, and Extracellular Matrix (ECM) for each person with a specific tumor

Under hypoxic conditions, TME demonstrates chemoresistance and reduces drug-induced cytotoxicity, which encourages cancer growth and spread.

A key factor in hypoxia-induced chemoresistance is the HIF protein. Under hypoxic conditions, Hif-1α encodes P-gp and increases the expression of MRP1, BCRP, and LRP. Additionally, Hif-1α supports DNA repair processes and inhibits the effects of chemotherapeutic drugs

According to research, MCF-7 cells are more resistant to the effects of chemotherapeutic medicines when the extracellular pH is lower

# Tumor microenvironment (TME)

The tumour microenvironment—the surrounding space composed of immune cells, stroma and vasculature—may mediate resistance by several mechanisms, including preventing immune clearance of tumour cells, hindering drug absorption and stimulating paracrine growth factors to signal cancer cell growth

Some immunotherapy-resistant tumours have a low mutational burden, which leads to a paucity of neoantigens that are available for presentation and ultimately prevents recognition

Immunosuppressive cancer microenvironments—so-called ‘immune deserts’—are now recognized as a major impediment to checkpoint inhibitors, owing to the presence of regulatory T cells, myeloid-derived suppressor cells, tumour-associated macrophages, cytokines and chemokines—all of which can inhibit immune-mediated anti-tumour effects.

This has led to the investigation of a range of techniques to turn immunologically ‘cold’ tumours into ‘hot’ ones by recruiting immune effectors.

# Cancer stem cells

- CSCs exhibit higher resistance to chemotherapies and radiotherapy than non-stem cells because of their dormant nature.
- Furthermore, CSCs are observed to have higher expression of anti-apoptotic proteins, low levels of reactive oxygen species, and greater efficiency in repairing DNA damage
- KLF4 is an important CSC transcriptional factor associated with increased carcinogenesis, stemness, and resistance to Adriamycin and Cisplatin in osteosarcoma
- KLF4 enhances chemotherapy resistance induced by high-mobility group box 1 (HMGB1) in osteosarcoma cells.
- Osteosarcoma patients are often treated with anticancer drugs like cisplatin, doxorubicin, and methotrexate.
- Elevated KLF4 levels increase HMGB1 expression through its binding and activation of the HMGB1 promoter.
- This heightened HMGB1 then binds to BECN1, promoting the formation of the PI3K III-Beclin 1 complex and facilitating autophagosome maturation, ultimately enhancing autophagy

# Cancer stem cells

One of the potential biomarkers for CSCs is aldehyde dehydrogenase (ALDH), which is a cytosolic enzyme that helps maintain low ROS levels inside the cancer subpopulation and interferes with subsequent death.

In ALDH-positive lung cancer, resistance to multiple chemotherapeutic drugs has been observed as compared to the ALDH-lung carcinoma patients

In the context of drug effluxion, CSC populations within various tumor types have been observed to exhibit elevated expression levels of ABC transporters, including P-glycoprotein (P-gp, MDR1, ABCB1), multidrug resistance protein 1 (MRP1, ABCC1), and breast cancer resistance protein (BCRP, ABCG2).

This heightened expression of ABC transporters confers a substantial degree of resistance to drugs

The WNT pathway is an upstream regulator of OCT4, and WNT-mediated activation of OCT4 is found to promote CSC subpopulations.

WNT-NOTCH crosstalk and β-CATENIN, downstream molecules in the WNT pathway, are also activated in high OCT4-expressing cancer cells.

Higher expression of OCT4 is associated with cisplatin, temozolomide, etoposide, doxorubicin, tamoxifen, paclitaxel, and gamma radiation-resistance in glioblastoma, breast and lung cancer