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# Overcoming Resistance:

## A Review on Chemotherapy Resistance in Cancer

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## Abstract

*Cancer has been around for as long as history can tell, and it is very prevalent in the United States today, affecting about one-third of people (The American Cancer Society Medical and Editorial Content Team, 2018). Traditionally, chemotherapy has been the method of treatment for cancer, inducing apoptosis and slowing down replication rate. Although successful in the past, cancer is becoming more and more resistant to chemotherapy treatment due to its ever-evolving nature. There are many mechanisms by which cancer evades chemotherapy, but four of the most common methods will be highlighted in this paper. Cancer cells are able to continue past cell cycle checkpoints and avoid apoptosis, which chemotherapy attempts to induce. The tumor microenvironment also plays a vital role in chemotherapy resistance, as does the presence of cancer stem cells. With all of these methods of evasion, there is room for new developments to treat cancer, including the use of CAR T-cell therapy, the use of ctDNA technology for earlier detection of resistance, optimizing doses, scheduling, and combination drugs, and implementing local delivery through ADCs. Although there are seemingly promising future therapeutics, further research must be conducted in order to find more solutions to resistance that specifically target chemoresistance.*

## Introduction

Cancer has been around for as long as humans are able to tell through recorded history. Some of the oldest findings of cancer have been in fossilized bone tumors, in human mummies of ancient Egypt, and in ancient manuscripts. An ancient Egyptian textbook on trauma surgery has the oldest written description of cancer. The text is from 3000 BC and describes different types of tumors of the breast. This was prior to the use of the word 'cancer.' The term was coined first by Hippocrates (460-370 BC), the Greek physician who is said to be the father of medicine. Hippocrates used the terms 'carcinos' and 'carcinomas'—words that mean crab in Greek. It is hypothesized that the use of a word meaning crab was due to the spreading of cancer resembling the shape of a crab. Carcinoma is now used to define a cancer that arises in the epithelial tissue of the skin or lining of the internal organs. The Roman physician Celsus (28-50 BC) translated carcino to cancer, which is the Latin word for crab, giving the widely used term today. Additionally, the Greek physician Galen (130-200 AD) used the word oncos in working with cancer, which is Greek for swelling. Today, this has evolved into the use of the term oncology, which is the study and treatment of tumors (The American Cancer Society Medical and Editorial Content Team, 2018).

Cancer is the second leading cause of death in the United States, and about one-third of all people in the U.S. will develop cancer in their lifetimes (The American Cancer Society Medical and Editorial Content Team, 2018). The first known method to treat cancer was by surgery to remove the tumors. When this didn't prove effective, radiotherapy was used. Eventually, and, traditionally, chemotherapy has been the best way to treat cancer, but resistance to chemotherapy has proven to be a large limiting factor in producing cures in

patients as of recent. The first solution to fight against this resistance was the use of polychemotherapy, which is the use of several different chemotherapy drugs that do not overlap in their mechanism. This method worked well in lymphoma, breast cancer, and testicular cancer (Vasan, 2019). After about 50 years of polychemotherapy working tremendously when in combination with surgery and radiotherapy, its efficacy plateaued. Targeted therapies seemed to be the next-best approach. With the understanding of the biological properties behind cancer came therapies with great efficacy. This, too, has seen problems in cancer evolving to promote its resistance. Following the same trend, immunological approaches using monoclonal antibodies that turn off the checkpoints of the adaptive immune system, and have proved effective, are now faced with resistance as well (Vasan, 2019).

Surgery → Radio-therapy → Chemo-therapy → Targeted Therapy → Checkpoint Inhibitors

*Figure 1: A timeline of the evolution of cancer treatments*

Chemotherapy resistance can be broken down into two main categories—acquired and intrinsic. Intrinsic resistance specifies that resistance factors existed in the tumor cells prior to the administration of chemotherapy drugs, rendering them ineffective. Acquired resistance, on the other hand, typically develops during treatments. In this case, tumors respond to treatment originally, but mutations and adaptive responses take over, allowing the cancer cells to evade the drug therapy. This is the typical route of resistance.

Additionally, tumors have a high amount of molecular heterogeneity, meaning that resistance can come about through selection of an originally small number of resistant cells (Holohan, 2013).

The size of tumors, in addition to the rate of tumor growth and changes in growth kinetics, play critical roles in responses to therapy and resistance as well (Vasan, 2019). Tumor heterogeneity is one of the most widely conceptualized methods of chemotherapy resistance. The processes that control tumor growth and survival are ever evolving and none is like the others, creating resistance mechanisms (Keller L. &., 2019). Exogenous exposures, internal environment, and the cancer therapies themselves can all lead to genomic instability, which is a driver for cancer to persist (Vasan, 2019).

Cancer is a dynamic, complex, ever-evolving problem, and the effectiveness of chemotherapy is widely limited by drug resistance. Here, I will attempt to explain the many ways that cancer is able to evade drug therapies, including the mechanisms used in evading cell repair and continuing past cell cycle checkpoints, how cancerous cells avoid apoptosis, the role of the tumor microenvironment in resistance, and how the presence of cancer stem cells help cancerous cells evade drug therapy. I will end by discussing some promising future therapeutics, although more research must be done in order to fully understand chemoresistance.

**Evading Cell Repair**

As stated previously, cancer is able to resist chemotherapy by four main mechanisms. The first of which is by evasion of cell repair. The cell cycle consists of the G1, S, G2, and

M phases. The G1 phase consists of preparing for synthesis, while the S phase is where DNA synthesis and chromosome duplication actually occur. Following the S phase, preparation for division occurs during the G2 phase. Mitosis, the division into two daughter cells, follows. G1, S, and G2 make up interphase in cells that are dividing continuously. Checkpoints exist before both the S phase and the M phase. In a typical cell, the cell cycle will arrest at these two points if DNA damage is present. At the first checkpoint, Retinoblastoma (Rb) acts as a brake, keeping the cycle in the G1 phase. Rb does this by inhibiting the genes that are necessary for entry into the S phase. When CDKs (cyclin-dependent kinases) are phosphorylated, the cycle can continue. If DNA damage is present, p53 stops the cell cycle here. Cancer has a way of evading these cell cycle arrests in order to continually proliferate.
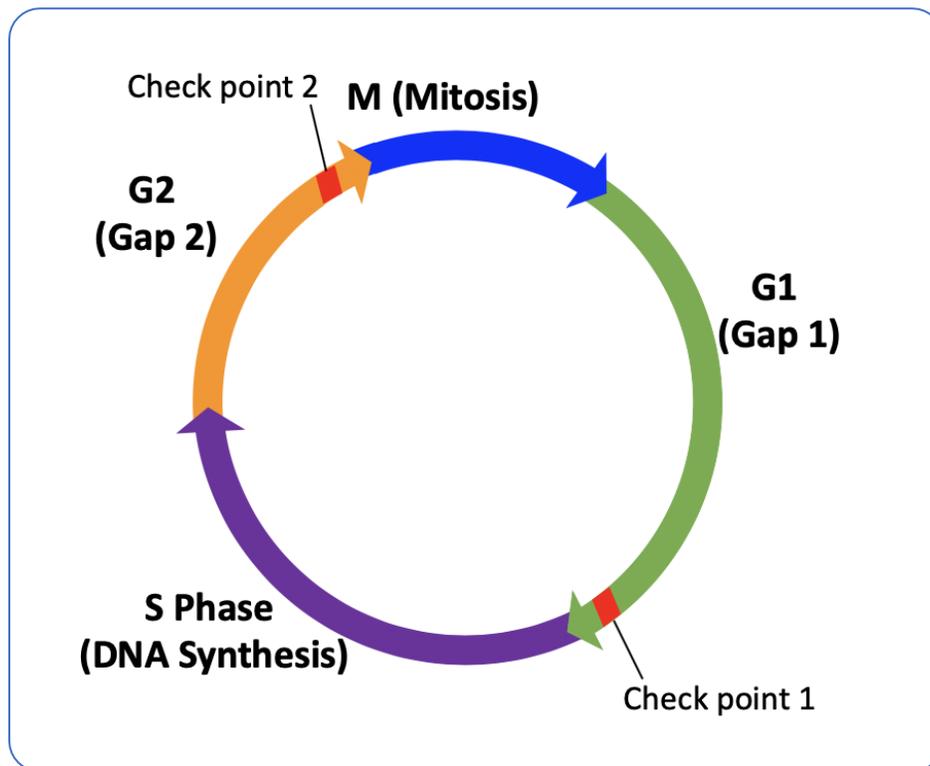


*Figure 2: A simple schematic of the cell cycle, including checkpoints 1 and 2, which play a role in chemotherapy treatment and in resistance.*

Traditional chemotherapy works by inducing DNA damage, which causes cell cycle arrest. If the damage is not able to be repaired in this arrest, cell death should ensue thereafter. Chemotherapy drugs can be cell cycle non-specific, meaning the drugs can kill at any point in the cell cycle, or they can be cell cycle-specific, meaning they kill cancer cells only during a specific phase in the cell cycle. These drugs induce DNA damage in hopes that the cell cycle will arrest and cause the cancerous cells to become apoptotic, thus getting rid of the cancer. One common way that cancer cells resist chemotherapy is by evading checkpoints—the cells continue throughout the cell cycle, making it past checkpoints that should otherwise stop them. This is due to either gain-of-function to oncogenes or loss-of-function to tumor suppressor genes. These act by disrupting the regulation of cell cycle arrest, such as by causing mutations in the p53 protein, which stops the cell cycle if there has been damage. The use of combination drugs, ones that both damage DNA and inhibit DNA damage repair, has been used to combat this.

Another mode of resistance in this realm is discussed in a paper by Jia et. al. on neoadjuvant endocrine therapy, which has been shown to be a useful treatment in ER-positive (estrogen receptor-positive) breast cancer patients, but resistance is still prevalent. In this study on neoadjuvant endocrine therapy (Jia, 2019), it was determined that RAD51 recombinase could potentially be a factor in the resistance. RAD51 is a major protein involved in homologous recombination, and it was found to interact with BRCA2. The enzyme encoded by RAD51 is involved in the repair of DNA double stranded breaks. Overexpression of RAD51 correlated with BRCA2 hypermethylation, resistance to therapy, and poor survival in patients (Jia, 2019). This ties into evasion of cell repair,

because dysregulation of the RAD51 gene is known to promote cell division and/or repress cell cycle arrest controls or apoptosis (Jia, 2019).

Another study that discusses this mechanism of resistance was conducted by Wang et. al. and looked at the LRH1 protein and the mismatch repair system. The mismatch repair system (MMR) is responsible for finding and repairing errors (insertions, deletions, incorrect bases) that occur during DNA replication. LRH1 (liver receptor homolog-1) has been shown to enhance tumor proliferation and development in human breast cancer (Wang, 2018). In this study, it was shown that knockout of LRH1 dramatically decreased non-homologous end-joining-mediated double-strand break repair efficiencies (Wang, 2018). In patients who had early recurrence in breast cancer, there were elevated levels of LRH1. This study (Wang, 2018) showed that LRH1 activates MDC1, a mediator of DNA damage checkpoint one. This activation, then, is able to further reduce the effects of DNA damage caused by chemotherapy. This reduction in DNA damage then causes the cancer to be able to evade the cell cycle checkpoints, further causing chemotherapy resistance.

## Avoiding Apoptosis

The second major mechanism of chemotherapy resistance includes that of avoidance of apoptosis. In the cell cycle, when DNA damage cannot be repaired, the cells undergo apoptosis—programmed cell death. In this case, the cell dies, the chromatin condense, the cytoplasm shrinks, and the membrane blebs. In cancer, this system fails. There are two pathways of apoptosis, the extrinsic pathway (death receptor-initiated pathway) and the intrinsic pathway (mitochondrial pathway). The extrinsic pathway is triggered by

receptor-ligand interactions, such as with the TNF (tumor necrosis factor) receptor family and the Fas family. This pathway activates caspase 8. The intrinsic pathway is initiated by injuries that cause mitochondrial permeability, which causes release of pro-apoptotic cytochrome c. This activates caspase 9. Caspase 8 and 9 both act on caspase 3, which initiates the effector stage of apoptosis, consisting of cleavage and inactivation of enzymes and structural constituents, fragmentation of genomic DNA, cytoskeletal breakdown, and the formation of apoptotic bodies.
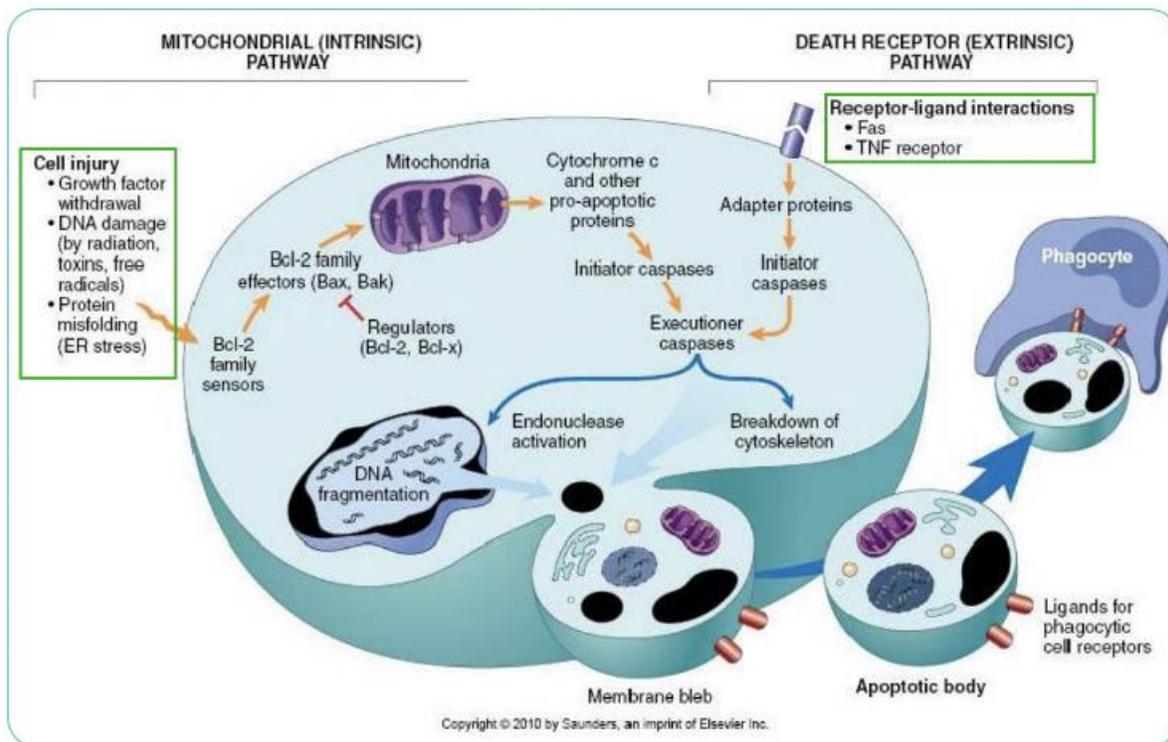


*Figure 3: A diagram of the two apoptotic pathways. The red line in the intrinsic pathway indicates inhibition. The caspase involved in the intrinsic is caspase 9, the extrinsic is caspase 8, and the executioner caspase is caspase 3* (Saunders, 2010).

The literature (Holohan, 2013) states that cancer cells are typically 'addicted' to a few anti-apoptotic proteins that promote their survival. The intrinsic, or mitochondrial, pathway relies on the pro- and anti-apoptotic BCL-2 family, and its members are the most prominent in those that cancer cells use to stay alive. Overexpression of the anti-apoptotic BCL-2 family members has been shown to cause chemotherapy resistance by blocking the intrinsic apoptotic pathway. This is indicated in *Figure 3* as the red inhibition arrow. Additionally, IAPs, or inhibitor of apoptosis proteins, have been linked to chemoresistance in patients (Holohan, 2013). IAPs act by blocking caspase 3, the caspase that causes the executioner phase. The executioner phase, as indicated above, causes the mechanisms of apoptosis, so, therefore, an abundance of them can cause avoidance of apoptosis. Finally, one more important player in avoiding apoptosis, is the caspase 8 inhibitor FLIP (Holohan, 2013). Caspase 8 is activated via the death receptor, or extrinsic, apoptotic pathway, which is shown on the right side of *Figure* 3. Inhibition of this caspase, then, would further inhibit the extrinsic apoptotic pathway from ensuing.

In a study by Arumugam et. al., overexpression of the GHR (growth hormone receptor) and its presence in ER-negative breast cancer was studied (Arumugam, 2019). In this study, the concentration of GHR was directly correlated to chemoresistance, metastasis, and tumor progression. Inhibiting GHR was linked to reducing the AKT/mTOR pathway. This intracellular signaling pathway is important in regulating cell metabolism, growth, proliferation, and survival. Inhibiting GHR in ER-negative breast cancer cells led to more apoptosis in the study, indicating that GHR plays a role in controlling the AKT/mTOR pathway, which then can impact chemoresistance. On the other hand, when GHR was

upregulated in the study, chemoresistance was also upregulated. This is yet another example of an upregulation in cancer cells that causes avoidance of apoptosis.

**Tumor Microenvironment**

The tumor microenvironment and the immune system have a large influence on the way tumors behave. In tumors that are solid, the microenvironment is composed of many cellular parts, including: the extracellular matrix, cancer-associated fibroblasts, immune and inflammatory cells, and blood vessels (Holohan, 2013). Recently, there has been much research conducted that focuses on understanding the immune system in the tumor microenvironment. Resistance due to the microenvironment has, in part, to deal with preventing immune clearance of tumor cells, causing a burden on drug absorption and prompting paracrine growth factors to signal cancer growth (Vasan, 2019).
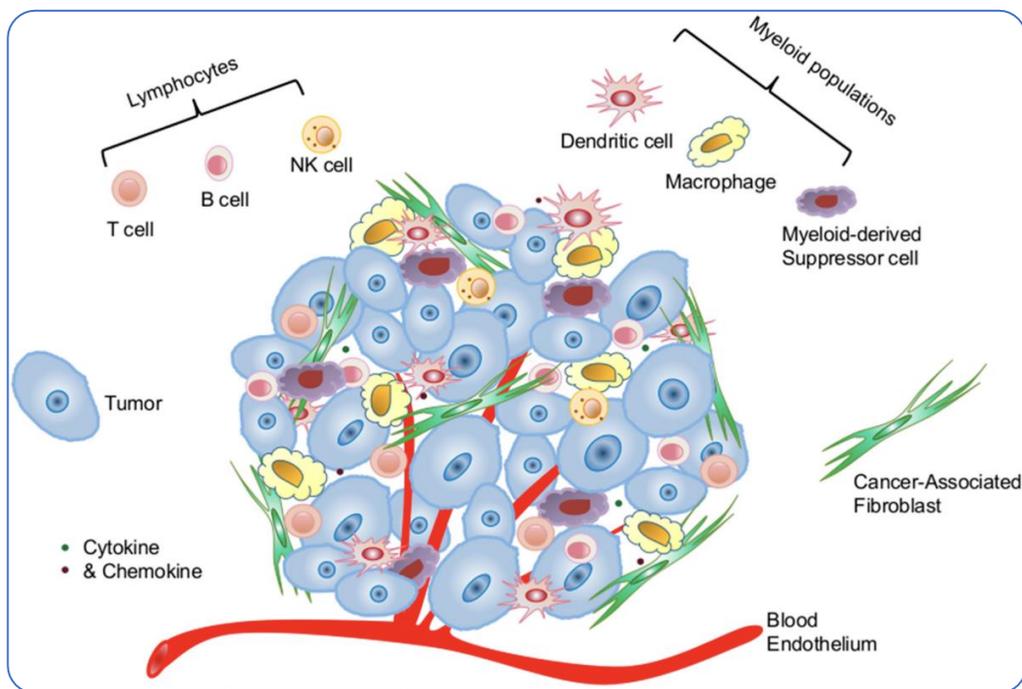


*Figure 4: A schematic of cellular components making up the tumor microenvironment, including immune cells and stromal cells* (Cui, 2016).

Additionally, the microenvironment provides protection for cancer cells to hide from cytotoxic agents. This is another way that cancer cells can evade apoptosis and acquire resistance to treatments. Integrins, cell surface adhesion molecules that connect cells to the extracellular matrix, play a vital role in resistance. Integrin expression is altered in tumor cells, and higher expression leads to increased cancer cell survival and drug resistance. Adhesion to the ECM via integrins has been shown to alter responses to chemotherapy in cancer by inhibiting apoptosis and altering drug targets (Holohan, 2013). Cytokines and growth factors play parts in resistance as well. These are able to maintain activation of pro-survival pathways, even in the presence of chemotherapy. This, then, relates back to avoidance of apoptosis and evasion of the cell cycle checkpoints.

In addition, spatial gradients of cancer cells within tumors due to the microenvironment can prevent enough blood flow, which can create a pro-tumorigenic hypoxic environment. This decreases the effective exposure of a tumor to drugs (Vasan, 2019). By similar mechanisms, cancer cells may take refuge in anatomical spaces where administered drugs are unable to reach them. One example of this would be cells colonizing in the central nervous system, allowing safety from drugs by the blood brain barrier.

The tumor microenvironment plays a vital role in hindrance to checkpoint inhibitors. Specifically, the regulatory T cells, myeloid-derived suppressor cells, tumor-associated macrophages, cytokines, and chemokines induce this inhibition (Vasan, 2019). With this hindrance, cancer cells are able to progress through the cell cycle, even with damage, as noted above. These parts of the tumor microenvironment can all inhibit immune-mediated and anti-tumor effects.

Finally, the epithelial-mesenchymal transition (EMT) can play a role in chemotherapy resistance in regard to tumor microenvironment. The EMT is the process in which tumor cells lose epithelial features (E-cadherin expression, epithelial cell junctions, etc.), and then develop mesenchymal characteristics. Epithelial cells lose their cell polarity and cell-cell adhesion and gain migratory and invasive properties to become mesenchymal stem cells. Due to this ability of the tumor cells to be in mixed states of epithelial and mesenchymal, the EMT has been linked to metastasis, but also to drug resistance (Sauvage, 2019).

## Cancer Stem Cells

The final mechanism by which cancer is able to avoid chemotherapy that will be discussed in this paper is that of the role of cancer stem cells.
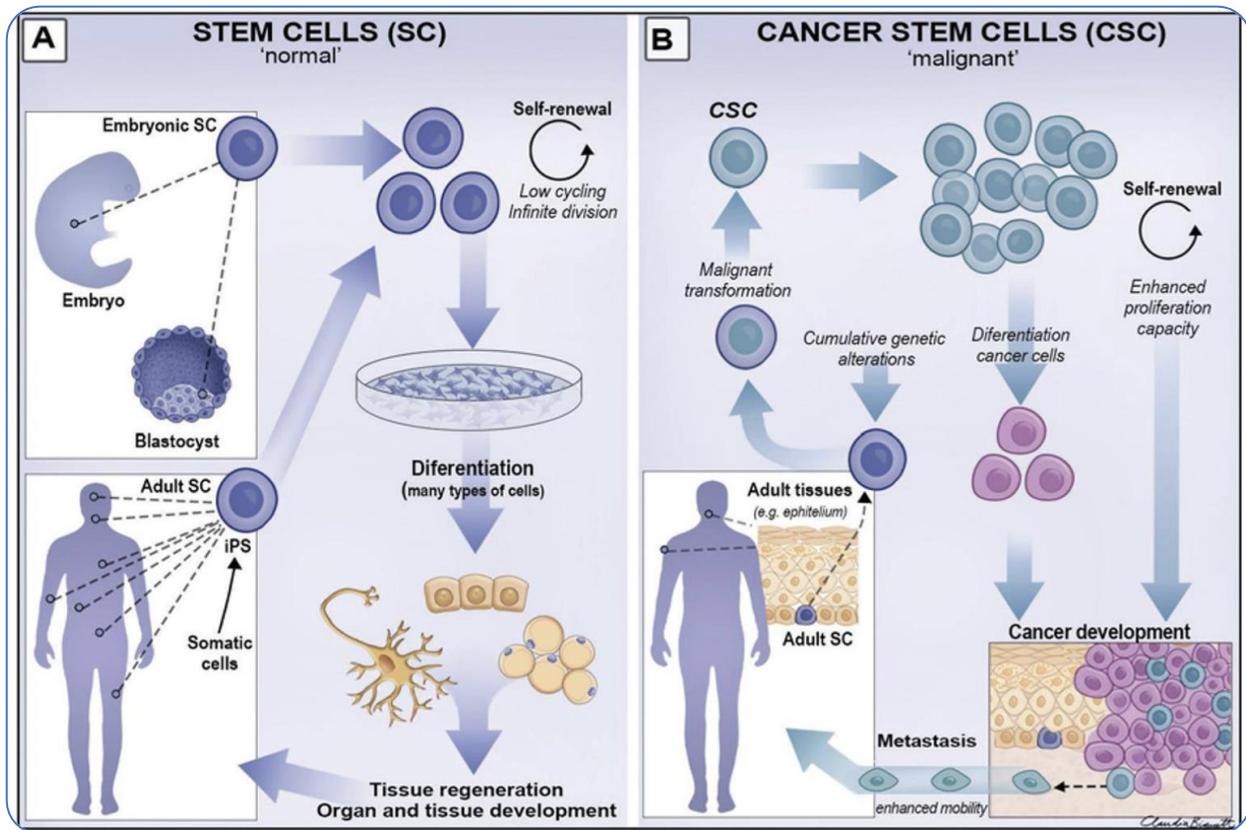
*Figure 5: The difference between normal stem cells and cancer stem cells. On the left, it is seen that normal stem cells come from a variety of tissues, and that they have indefinite division through self-renewal and generation of differentiated cells under appropriate conditions. On the right, adult stem cells can undergo malignant transformation after cumulative genetic alterations caused by carcinogens, generating CSCs. These CSCs retain the biological properties of the self-renewal and generation of differentiated cancer cells, leading to cancer development and further metastasis (Rodini, 2017).*

Cancer stem cells have been linked to acquired chemotherapy resistance in many different types of cancers. Cancer stem cells (CSCs) have many properties that other cells lack, allowing them to evade chemotherapy. CSCs have the ability to self-renew,

promoting the recurrence of tumors after chemotherapy. Other studies have shown that CSCs do not have differentiation markers (Vidal, 2014). The maintenance of the non-dividing state during the cell cycle while still having the ability to reenter the proliferative part of the cell cycle is called quiescence. This is a very important feature of CSCs, indicating that many of them can lie "dormant" and appear as if the cancer is gone in patients but can jump right back into the proliferative stage at any point (Vidal, 2014).

DNA methylation, RNA-mediated targeting, and histone modification are epigenetic mechanisms by which gene expression can be altered. They also regulate many other important biological processes in cancer. CSCs are suspected to be genetically identical to their differentiated cells, with epigenetic regulators fundamental to the CSC state (Vidal, 2014). As was already discussed previously, cancer cells resist chemotherapy by evading apoptosis and promoting cell survival. It is believed that cancer stem cells play a pivotal role in this resistance. They have been shown to deregulate mitochondrial proteins, decrease DR5 (death receptor 5) expression, interact in cytokine signaling, and develop signaling pathways that promote survival (Vidal, 2014). Additionally, it has been discovered in recent studies that CSCs have distinct metabolic activities. This can be linked to the uncontrolled proliferation of cancer cells due to problems with the regulation of metabolism. Finally, the epithelial mesenchymal transition, can also be important in CSC mechanisms to resist chemotherapy. The EMT is an important regulator of the CSC phenotype and tumor heterogeneity, which both relate back to resistance mechanisms (Shibue T. &., 2017).

With all of these mechanisms highlighted, it can be observed that many of the mechanisms of chemotherapy resistance in cancer stem cells are the same mechanisms responsible for chemotherapy resistance in normal cancer cells, just with a unique perspective toward cancer stem cells.

## Future Therapeutics

Chemotherapy resistance is a grand problem that is very prevalent. It occurs by many mechanisms, but those mechanisms can mostly fall into four main categories: evading cell repair, avoiding apoptosis, factors due to the tumor microenvironment, and the presence of cancer stem cells. This list is in no way comprehensive, but hits on the major methods known today of chemotherapy resistance. Each of these categories has complex mechanisms by which they cause resistance, and each deserves further research into how best to deal with resistance moving forward—most importantly, how to avoid it.

Many of the mechanisms of chemotherapy resistance can be targeted by specific therapies with the intent of destabilizing these mechanisms. For example, the use of CAR T-cell therapy has shown promising preliminary results as a cancer therapy. This therapy is a form of immunotherapy that uses specially altered T cells to fight the cancer. In this therapy, a sample of a patient's T Cells are collected and modified to present chimeric antigen receptors (CARs) on their surface to aid in therapy. Cancer stem cells can specifically be targeted to inhibit their resistance mechanisms as well. Their self-renewal, lack of differentiation, and quiescence can be specifically targeted.

Tumors are typically detected as being resistant simply through biopsy techniques, but more in-depth methods would be able to identify resistance earlier, especially since different metastases can have different resistance mechanisms. There is promise in using ctDNA technology to also help in this aspect, with the ability to monitor heterogeneity, evolution, and response to therapy (Vasan, 2019). Circulating tumor DNA (ctDNA) can be detected in the blood, and testing for it can be done to find cancers early (Vasan, 2019). Circulating tumor DNA is tumor-derived fragmented DNA in the bloodstream. Using ctDNA-based methods could provide monitoring of tumors in real-time, allowing providers to be able to identify patients at risk of relapse and to aid in the planning of the next modes of therapy based on tumor behavior. The use of ctDNA as a screening tool is a new idea, so clinical trials and further research must be conducted on its efficacy before being implemented.

In the interest of achieving deeper responses to chemotherapy, optimizing doses, scheduling, and combination drugs/therapies is vital. These must all be adapted together as studies have shown that just increasing dose alone, for example, does not promote tumor response to the drug. Another way to increase response would be to implement local delivery of chemotherapy—a dose that would be too toxic if delivered systemically, but that, when given locally, targets the tumors well. This has been shown to be effective through ADCs (antibody-drug conjugates). These are recombinant monoclonal antibodies covalently bound to cytotoxic agents through synthetic linkers. ADCs can deliver large amounts of the drugs directly to the tumor cells, since the antibodies can be engineered to bind to antigens that are exposed on tumor cells with very high affinity (Vasan, 2019).

Finally, research needs to be furthered in order to be able to fully understand the dependencies of cancer. With this knowledge, therapies would be able to specifically combat certain chemoresistance mechanisms in order to have the best outcomes for patients.

**Conclusion**

Resistance to chemotherapy is the biggest challenge to fighting cancer today. Cancer resists chemotherapies by many different mechanisms, some of which have yet to even be discovered. Through extensive research, it has been found that cancer resists chemotherapy by four main mechanisms, which encompass the main ideas about resistance: evading cell cycle checkpoints, avoiding apoptosis, tumor microenvironment factors, and the presence of cancer stem cells. These mechanisms all stem from the plasticity and heterogeneity of cancer cells. Knowing that each cancer is infinitely unique presents many challenges in terms of trying to find a cure for chemotherapy resistance. Current research has found a few ideas that seem promising to these problems, including the use of ADCs for specific targeting and ctDNA for detection and real-time monitoring. Chemotherapy resistance is a complex issue that is very prevalent in the community today, and in order to further understand it and put a stop to it, more research must be done.

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