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Multi-Drug Resistance in Cancer: Understanding of Treatment Strategies

Rishabha Malviya*, Arun Kumar Singh and Deepika Yadav

Abstract

In the United States, cancer is the second largest killer. Surgery, cytotoxic chemotherapy, targeted therapy, radiation therapy, endocrine therapy, and immunotherapy are among the most important therapies for cancer management. Resistance to conventional chemotherapeutic agents and/or innovative targeted medications remains a significant challenge in cancer treatment despite decades of effort and progress. Cancer relapses are a leading cause of mortality, and they are often the result of either preexisting drug resistance (intrinsic) or the development of new drug resistance (acquired). Drug resistance is more difficult to manage due to the heterogeneity of people and tumors, as well as cancer's adaptability in evading therapy. To better direct future cancer therapy and boost outcomes, a better understanding of the factors that contribute to drug resistance is required. In this synopsis, examination of both innate and acquired forms of resistance. In addition, new information about the mechanisms of drug resistance will be presented and discussed. The details of recent findings that highlight the role of ATP in drug resistance, including the presence of extremely high levels of extracellular ATP within tumors and the importation of extracellular ATP into tumor cells from the environment has been discussed here. Due to the complex nature of drug resistance, it is possible that combining and customizing treatments for cancer patients may be the most effective strategy for combating the disease.

***Keywords*:** Cancer, drug resistance, heterogeneity of tumor, combination therapy, acquired resistance

1.1 Introduction

To put it simply, cancer is the second largest killer in the United States [1]. A total of 1.7 million persons were diagnosed with cancer in 2017,

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with 0.6 million succumbing to the disease [2]. Up to 90% of cancer-related fatalities are caused by medication resistance and the ensuing ineffectiveness of treatment [3–7]. The mechanism of drug resistance against cancerous cells is illustrated in Figure 1.1.

Cancer cells that have acquired tolerance to pharmacological therapy are said to have developed drug resistance. Many different physiological and molecular pathways, including mutations and epigenetic alterations in cancer cells, upregulation of a previously conserved drug efflux pump, and others, contribute to the development of resistance to anticancer treatments.

Surgical resection, cytotoxic chemotherapy, targeted therapy, radiotherapy, irradiation, hormone therapy, and immunotherapy are now the cornerstones of cancer management [8–12]. Although there have been great strides achieved in cancer treatment over the last several decades, relapses a leading cause of cancer deaths are still often the result of the body's resistance to chemotherapy or targeted medications. Many traditional chemotherapeutic anticancer medicines work by directly altering the DNA of cancer cells to destroy them. This method is inherently non-specific and

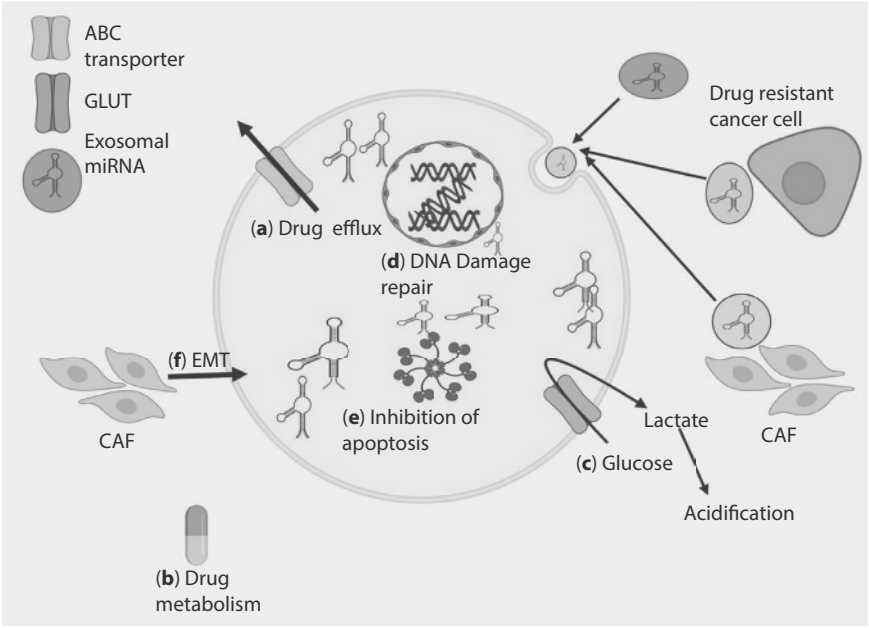

Figure 1.1 Diagrammatic representation of cancerous cells developing resistance against the drug.

may have severe side effects in certain patients. Many new medications that specifically target or inhibit cancer-promoting alterations have been produced in the last few decades. While the earliest stages of therapy with these medications may have spectacular outcomes, the vast majority of patients will eventually develop resistance. To provide just one example, between 30% and 55% of individuals with non-small cell lung cancer (NSCLC) have a recurrence and ultimately succumb to the disease [13]. Recurrence of ovarian adenocarcinoma is common, occurring in 50% to 70% of cases within a year following surgery and chemotherapy [14]. About 20 % of juvenile acute lymphoblastic leukemia patients suffer recurrence [15].

Better knowledge of the processes behind the formation of drug resistance is critically required and will help to develop innovative treatment options and lead to better clinical results. This chapter will explain what drug resistance is and how it may be acquired or developed, as well as highlight recent findings on the causes of drug resistance and address novel approaches to combating drug resistance and enhancing the effectiveness of anticancer drugs.

1.2 Both Congenital and Developed Resistance to Drugs

The development of drug resistance may be classified as either innate or acquired. About half of all cancer patients with drug resistance have intrinsic resistance, which occurs before medication treatment, and 50 % have acquired resistance, which is produced by therapy [16, 17].

1.2.1 Intrinsic Resistance

For medications to have less of an effect, they must overcome the patient's natural resistance to them, which is known as "intrinsic resistance." (1) Preexisting (inherent) genetic mutations in the majority of tumors lead to decreased responsiveness of cancer cells, such as triple-negative breast cancer cells, to both chemo and target drugs; (2) heterogeneity of tumors in which preexisting insensitive subpopulations, including cancer stem cells, will be selected upon drug treatment, resulting in a relapse in later stages of treatment; (3) activation of intrigue resistant pathways (such as anticancer drugs).

Preexisting genetic mutation(s) of genes involved in cancer cell proliferation and/or apoptosis may cause cancer cells to be intrinsically resistant to drugs. Overexpression of HER2 has been linked to a worse response to

cisplatin in patients with gastric cancer [18]. Increased resistance to chemotherapy is achieved by a process called epithelial-mesenchymal transition (EMT), which is triggered by increased HER2 gene expression by upregulating the transcription factor Snail. It was also discovered that the survival rate of HER2/Snail double-positive patients was lower than that of single-positive or double-negative patients.

EMT, resistance to p53-induced apoptosis, and a self-renewal drive were all demonstrated to be mediated by the transcriptional repressors Snail and Slug [19]. These two processes provide radiation and chemotherapeutic resistance to cancer stem cells (CSCs). Resistant cells have also been demonstrated to have greater mesenchymal characteristics [20, 21]. These parallel alterations establish a connection between inherent drug resistance, EMT, and CSCs.

Relapse after chemotherapeutic therapy may also be caused by preexisting resistant subpopulations in malignancies. Increasing data indicate that intratumoral genetic variability in primary cancers exists prior to therapeutic intervention [22–24]. The majority of tumor cells are vulnerable to the medicine; therefore, patients would initially react to treatment. After pharmacological therapy, however, the resistant subclones would multiply and produce a relapse [25–27]. This inherent drug resistance is sometimes misunderstood as acquired resistance since the tumor would initially shrink during treatment, leading many to believe that the resistance was gained as a result of therapy. Cancer stem cells (CSCs) are a self-renewing and differentiating subset of tumor cells that contribute to tumor development [28]. Multiple cancer types, including leukemia [29], glioblastoma [30], and pancreatic cancer [31], have documented their involvement in chemotherapeutic treatment resistance. It is possible that medication resistance may only be mitigated by the use of a combination treatment that simultaneously eliminates CSCs and the bulk of the tumor.

Anticancer medications are no exception; activation of intrinsic mechanisms intended to defend against environmental contaminants might decrease their therapeutic effects. ATP binding cassette (ABC) transporter-mediated drug efflux [32] and the glutathione (GSH)/glutathione S-transferase system are examples of such defence systems that operate to either decrease drug accumulation in cells or detoxify drug-treated cancer cells, respectively [33].

1.2.2 Acquired Resistance

Slowly diminishing anticancer activity after pharmacological therapy is a hallmark of acquired resistance. Tumor microenvironmental (TME)

alterations, mutations in therapeutic targets, and secondary oncogene activation are all potential causes of acquired resistance.

Tumors that have been reduced in size may regain growth capability if they develop resistance to treatment. Eight patients with acute myeloid leukemia had their genetic profiles examined using whole-genome sequencing before and after relapse [34]. Genome-wide analysis of primary and recurrent cancers revealed previously unknown alterations in genes. Furthermore, the data demonstrated an increase in transversion mutations in recurrent tumors, which may indicate that DNA damage in cancer cells produced by cytotoxic chemotherapeutic treatments enhanced the likelihood of the development of new mutations.

When mutations or changes in the expression levels of the genes-producing target proteins occur in cancer cells, the cells may become resistant to the therapeutic effects of the medications. As an example of a secondary mutation inside the target kinase, the threonine 315 to isoleucine (T315I) mutation in the BCR-ABL kinase domain is a case in point. Although the BCR-ABL tyrosine kinase inhibitor (TKI) imatinib is widely used to treat chronic myelogenous leukemia, between 20% and 30% of individuals develop resistance to the drug or relapse following treatment [35]. The resistance may be explained, in part, by a point mutation in the fusion tyrosine kinase protein, T315I, which prevents it from functioning properly [35–37]. By replacing threonine 315 with isoleucine, the drug's effectiveness is greatly diminished because the ATP-binding site of BCR-ABL no longer forms a hydrogen bond with imatinib.

The dynamic variations in TME during therapy may potentially lead to the development of drug resistance. Tumor cells and their microenvironment engage in cross-talk throughout disease progression and resistance. The interaction involves exosomes secreted by both cancer and stromal cells. Cancer cells and tumor-associated macrophages (TAMs) in the TME rely on exosomes, which are generated by cancer cells and contain specific miRNAs, to interact with one another, according to the research [38]. Neuroblastoma (NBL) tumor cells secrete exosomic miR-21, which stimulates tumor-associated macrophages (TAMs) to generate exosomic miR-155, which then silences the TERF1 gene in NBL cells. Increased telomerase activity and tolerance to chemotherapy would arise from reduced expression of TERF1, a protein that inhibits telomerase. As a result, drug resistance may be facilitated by the exchanging of exosomal miRNAs between tumor cells and stromal cells in the TME.

While tumors develop and are treated, the innate and acquired resistance mechanisms discussed above might coexist. There may be significant differences between the underlying mechanisms of intrinsic drug

resistance and those of acquired drug resistance. Also possible is a gradual increase in the organism's innate resistance to drugs. The susceptibility of individual cancer cells to a certain treatment is determined beforehand by the extent to which those cells exhibit inherent drug resistance. It is important to rule out the possibility of medication resistance by conducting genomic and other biochemical tests before designing the treatment strategy. Adjustments to treatment plans are necessary when acquired medication resistance has emerged.

One goal of cancer medication therapy should be to halt tumor development without triggering acquired, or at least unmanageable, drug resistance. Acquired drug resistance is a serious problem that has to be addressed in any effective drug treatment approach.

1.3 Drug-Resistance Mechanisms

Although differentiating between innate and acquired resistance is vital from a scientific standpoint, the particular mechanisms of resistance are more relevant from a therapeutic perspective.

1.3.1 Increased Efflux of Drugs

It has been hypothesized that reduced intracellular drug accumulation as a result of increased anticancer agent efflux is the primary cause of chemotherapy resistance [7, 39, 40]. Excessive drug efflux rates may indicate innate or acquired resistance, depending on whether the problem appears before or after drug treatment.

The ABC transporter superfamily is the most common source of transmembrane transporters involved in drug efflux. There are 48 ABC genes in the human genome, and these genes have been divided into seven different subfamilies (ABCA-ABCG) [41, 42]. Among them, ABCB1, ABCC1, and ABCG2 play significant roles in the development of MDR to cancer chemotherapy.

Too far, ABCB1 (also known as MDR1 or P-gp) has been one of the best-studied ABC transporters. It consists of two nucleotide-binding domains that bind and hydrolyze ATP and two transmembrane domains that establish a passage for substrates. Transport substrates are pumped out as a result of conformational changes in the transporter that occur in conjunction with ATP binding and hydrolysis [43]. Etoposide, doxorubicin, paclitaxel, and vinblastine are only some of the many substrates that may be bound and pumped out of the cell by ABCB1 due to its many drug

binding sites [44–48]. Numerous tumor forms, including kidney, lung, liver, colon, and rectum, have been shown to display high levels of ABCB1 prior to chemotherapy [49]. In contrast, numerous hematological malignancies, including AML and ALL, showed initially modest expression of ABCB1, followed by a substantial rise in expression of ABCB1 after chemotherapy [50–52].

ABCC1, also known as multi-drug resistance-associated protein 1 (MRP1), is responsible for the secretion of several different classes of anticancer drugs, including vinca alkaloids, anthracyclines, epipodophyllotoxins, camptothecins, and methotrexate [53]. Transported by ABCB1 include amphipathic and lipid-soluble molecules, whereas organic anionic substrates such as those conjugated to glutathione, glucuronide, or sulphate are pumped by ABCC1 [54–56]. ABCC1 overexpression has been linked to resistance in a variety of malignancies, including lung, breast, and prostate cancers [53, 57, 58].

In breast cancer, the protein ABCG2 (breast cancer resistance protein) is the primary drug efflux transporter responsible for the disease's resistance to treatment. Some malignancies have been linked to ABCG2, a gene responsible for the so-called bystander population impact. A wide variety of medicines, including chemotherapeutics (mitoxantrone, bisantrene, epipodophyllotoxin, camptothecins, flavopiridol, and anthracyclines) and TKIs (imatinib and gefitinib), are transported by this transporter [48, 59, 60]. Overexpression of ABCG2 has been identified in a wide variety of cancers, including breast cancer, lung cancer, and leukemia [60, 61].

Additional insights into the mechanisms of drug resistance have been gleaned from studies of the substrates and activities of other ABC transporters involved in tumor resistance to anticancer drugs [62]. Overexpression of ABC transporter genes, such as ABCC2 and ABCC3, causes multi-drug resistance [62–65]. These genes are responsible for transporting a wide variety of chemotherapeutic medicines, including cisplatin, doxorubicin, and etoposide. ABC transporter mutations and overexpression have a direct impact on tumor sensitivity and the therapeutic value of anticancer medicines. Better medication selection and treatment results need an accurate and comprehensive expression profile of ABC transporters in malignancies.

1.3.2 Impact on Medication Target

Targeted treatments may impede the growth of cancer cells by blocking the action of particular target proteins involved in tumor formation, making them more selective and effective to cancer cells and less destructive to

normal developing cells than typical chemotherapies. However, resistance may also emerge as an issue with targeted treatment, as a consequence of changes to medication targets. Secondary mutations in the target protein or changes in expression levels as a result of epigenetic modifications are two possible causes of medication target changes.

The epidermal growth factor receptor (EGFR) TKIs erlotinib and gefitinib, which are used to treat NSCLC, have reportedly shown a high response rate at the start of treatment [66, 67]. However, within a year, over half of the responding patients would have a T790M mutation on EGFR, leading to resistance to the first and second generations of TKIs [68–70]. The alteration in EGFR conformation brought about by the threonine-to-methionine mutation increased ATP binding affinity and decreased gefitinib/erlotinib binding to the kinase [70, 71]. Third-generation TKIs, such as osimertinib and rociletinib, have been developed and shown to have therapeutic effectiveness with patients having the T790M mutation [72, 73], hence overcoming the resistance conferred by this mutation. Resistance to third-generation inhibitors develops quickly, however, therefore developing fourth-generation TKIs is essential. The C797S mutation in EGFR has been identified as a potential mechanism of the novel resistance [74]. The binding of third-generation TKIs to EGFR is hindered by the absence of the cysteine residue, which is critical for TKIs to target the ATP site. As a result, EAI045, a fourth-generation TKI that targets both T790M and C797S, was developed to bind an allosteric site on EGFR to avoid the mechanism patterns of resistance seen with the first three generations of TKIs, all of which bind to the ATP sites [75, 76]. In the never-ending fight against drug resistance, a new trend may emerge the competition between the creation of new genetic mutations and the creation of new TKIs that restore drug sensitivity.

Such a case in which resistance is produced by a change in the therapeutic target is the creation and usage of oestrogen receptor inhibitors in the treatment of breast cancer. Commonly prescribed to those with ER-positive breast cancer, tamoxifen (TAM) works by competing with oestrogen for the ligand-binding site of ER. However, medication resistance is often developed after prolonged exposure to TAM. Mutations in the ER gene and decreased ER expression levels are two examples of resistance mechanisms that may occur in individual cases [77, 78]. Aromatase inhibitors (AIs) were created as a solution to the issues with TAM and the need for new medications. These treatments act by blocking the last stage in the production of oestrogen. In postmenopausal women with hormone receptor-positive breast cancer, third-generation AIs are increasingly employed as first-line therapy [79].

1.3.3 Improved DNA-Damage Repair

Chemotherapy medications, such as cisplatin and 5-fluorouracil (5-FU), cause cancer cells to die through DNA damage. Due to DNA lesion repairs, afflicted cells' DNA damage response (DDR) to anti-cancer treatments may impair the medications' effectiveness, leading to resistance [80]. For instance, 5-FU–resistant human colon cancer cell lines were discovered to have an upregulation of DNA repair genes, such as FEN1, FANCG, and RAD23B [81, 82]. Treatment with 5-FU led to the overexpression of genes involved in DNA damage response and repair that are p53 target genes. Cell cycle arrest and apoptosis were at lower levels in resistant cell lines compared to parental cell lines due to the successful healing of the damage [82].

While DNA damage response (DDR) downregulation might alleviate resistance caused by DNA repair, it also raises the prospect of acquiring additional mutations owing to genomic instability, the accumulation of which can spark a new cycle of carcinogenesis. As a result, the DNA damage response is a mechanism involved in cancer therapy and recurrence that needs careful examination before being employed as a therapeutic target in the fight against cancer.

1.4 Senescence Escape

Cellular senescence is defined as the permanent halt in cell growth that often results in the activation of tumor suppressive mechanisms controlled by p53 and/or p16INK4a [83]. Excessive mitogenic signaling from activated oncogenes, telomere shortening [84], and non-telomeric DNA damage from chemotherapeutic medicines are all important triggers that may induce cellular senescence. Chemotherapy drugs like doxorubicin and cisplatin, for instance, cause cell death and may also induce senescence [85, 86].

As previously mentioned, drug resistance and tumor recurrence/progression might occur as a result of tumor cells evading senescence induced by treatment (TIS) [87]. By acquiring stem-cell characteristics, cancer cells with TIS can avoid senescence and recurrence [88, 89].

1.5 Epigenetic Alterations

Epigenetic changes are an emerging mechanism that contributes to medication resistance. There is growing evidence that epigenetic alterations

have a role in the development of resistance mechanisms such as improved drug efflux, accelerated DNA repair, and defective apoptosis [90–93].

DNA methylation, histone modification, chromatin remodeling, and changes to non-coding RNAs are all examples of epigenetic modifications [94]. Increased expression of an oncogene, for instance, would arise from demethylation of DNA in the gene's promoter region, which might lead to resistance to treatment. Recent research showed that in a resistant hepatocellular carcinoma (HCC) cell line, the G-actin monomer binding protein thymosin 4 (T4) was enriched by demethylation of DNA and active modification of histone H3 at the promoter region [95]. The HCC cell line acquired stem cell-like properties with T4 overexpression, and the cells were resistant to the VEGFR inhibitor sorafenib *in vivo* [95].

Non-coding RNAs, including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), have a significant role in drug resistance [96, 97], in addition to chromosomal alteration. LncRNAs may be anywhere from 200 to over 10,000 nt in length, whereas miRNAs only have approximately 21 to 25 nt. As a result of binding to their corresponding mRNAs, miRNAs mediate mRNA degradation and suppress protein synthesis, earning them a prominent role as regulators of post-transcriptional gene expression. By inhibiting the binding of transcription activators to critical DNA regions in genes and by attracting chromatin remodeling proteins, LncRNAs play a role in the control of gene expression. The expression of proteins involved in cancer medication resistance is controlled by both microRNAs and long noncoding RNAs. It has been demonstrated, for instance, that cisplatin-resistant bladder cancer cells express higher levels of the lncRNA urothelial cancer-associated 1 (UCA1) than susceptible cells [98]. Increasing mRNA and protein levels of wingless-type MMTV integration site family member 6 (Wnt6) was shown to promote Wnt signaling and cell viability when UCA1 expression was upregulated [98].

1.6 Tumor Heterogeneity

Tumors exhibit four types of heterogeneity: genetic, cellular (cancer cells, stromal cells, immune cells, etc.), metabolic (oxygen/nutrient distribution), and temporal (dynamic tumor progression) [99]. Because of tumor heterogeneity, it is almost hard to eradicate all cancer cells with a singular therapy. Combinational therapy, such as FEC (5-fluorouracil, epirubicin, cyclophosphamide) for breast cancer, was developed as a solution to this issue. Here, the authors will discuss about the phenomenon of genetic diversity.

Primary tumors of several cancer types, including ovarian cancer [100], renal cell carcinoma [101], breast cancer [102], and chronic lymphocytic leukemia [103], have been demonstrated to house distinct subpopulations of cancer cells with distinct genetic profiles. Variable clonal variations of a tumor have different sensitivities to chemotherapy and targeted medications, meaning that early treatment can only eradicate some of the tumor while leaving others to persevere. If the resistant clones continue to multiply and expand, the tumor will reappear but this time with a new cell composition that is resistant to the original treatment. Evidence that the subclonal compositions vary dramatically at various periods of therapy [23, 24, 101, 104] lends credence to the idea that genetic heterogeneity of the subpopulations develops during drug treatment in a Darwinian selection way. Exosome-mediated transfer of microRNAs from drug-resistant tumor cells to drug-sensitive tumor cells may cause resistance in both types of tumor cells in heterogeneous tumor cell populations [105].

Studies documenting the decline in responsiveness to targeted medications provide credence to the idea that tumor heterogeneity plays a role in the development of treatment resistance. Targeted medicines have the potential to increase effectiveness and decrease adverse effects, but their high specificity may become a drawback when confronting tumor heterogeneity. Therefore, multi-drug combination therapy is necessary to either prevent tumor recurrence entirely or significantly slow its progression.

And since different individuals may react differently to the same medication, research into customized medicines is critically needed.

1.7 Tumor Microenvironment

Different cell types and the extracellular matrix (ECM) all have a role in the development, progression, and metastasis of tumors [106, 107]. The microenvironment of solid tumors consists of an extracellular matrix (ECM), immunological and inflammatory cells, blood arteries, fibroblasts, and numerous nutrients and signaling chemicals. Together, they play crucial roles in tumor development and survival.

TME has been linked to cancer's innate resistance to treatment. The TME variables include pH. The pH outside of healthy tissue or cell is somewhat higher than the pH inside of it (pHe7.3–7.5 vs. pHi6.8–7.2) [108]. However, cancer cells raise internal pH and decrease external pH by pumping protons through proton transporters and modulating pH sensors [109, 110], creating a so-called reversed pH gradient. According to some research [111], cancer cells' extracellular environment, which is often

acidic (pH 6.5–7.1), may contribute to their resistance to chemotherapeutics. Cancer cells are able to avoid apoptosis because the distribution of weak base anticancer medications is hampered by the inverted pH gradient, a phenomenon known as "ion trapping" [112, 113]. The low extracellular pH that is increasingly being seen in solid tumors is a novel signature of these diseases that might be targeted in cancer treatment. Proton pump inhibitors (PPIs) and other therapeutic strategies that aim to lower the acidity of the microenvironment have been developed and proved to be effective in reducing tumor size and making cancer cells more sensitive to chemotherapy. One proton pump inhibitor (PPI) that has been shown to work synergistically with paclitaxel in melanoma cells *in vitro* and *in vivo* is lansoprazole [114].

Adaptation of cancer cells to chemo or targeted treatments, which reduces drug effectiveness and induces resistance, is also facilitated by post-treatment changes in the composition of TME. For instance, in the case of glioblastoma multiforme (GBM), a particularly deadly kind of brain tumor, TAMs contribute to the development of resistance to anticancer therapies [115]. To aid cancer cell growth and survival, macrophages in GBM tumors release large amounts of colony-stimulating factor-1 (CSF-1) [115, 116]. Cancer therapy that employs small molecule inhibitors or antibodies against CSF-1 receptor (CSF-1R) has shown encouraging *in vivo* effects [117–119]. Increased insulin-like growth factor-1 (IGF-1) production from TAMs and IGF-1–induced increase of phosphatidylinositol 3-kinase (PI3K) pathway signaling in glioblastoma multiforme (GBM) tumor cells contribute to the recurrence of GBM in over 50% of patients [115]. It has been shown in animal models that blocking both the CSF-1R and the IGF-1 receptor or PI3K signaling together increases survival time. Therefore, combination treatments that concurrently target cancer cells and TME may result in significantly increased anticancer effectiveness by lowering drug resistance.

Furthermore, TME heterogeneity enriches genetic heterogeneity, which is itself a facet of tumor heterogeneity. Variable hypoxia is one feature of the TME [120], which occurs because tumor vasculature is variable and dynamic. Oxidative stress caused by repeated hypoxia and reoxygenation may cause DNA damage in tumor cells, leading to an increase in mutations and the emergence of genetically distinct clonal subpopulations [121]. Further, as was previously indicated, cells in the TME, such as TAMs, contribute to tumor heterogeneity by interfering with the expression patterns of cancer cells by the release of exosomes carrying miRNAs [38].

As a result, TME is very important in tumor development and resistance to treatment. A more complete understanding of the TME and its

interaction with tumor cells might significantly improve therapeutic response and clinical outcomes.

1.8 Epithelial to Mesenchymal Transition

The epithelial-to-mesenchymal transition (EMT) is a process in which epithelial cells change into mesenchymal stem cells by detaching from one another. While it is well established that EMT is necessary for the development of metastasis in cancers of epithelial origin, its involvement in other malignancies, such as sarcomas, is less well understood. There is mounting evidence that EMT is a key player in the development of resistance to chemotherapy. According to research conducted on an EMT lineage-tracing mice model by Fischer *et al.*, EMT enhances resistance to apoptotic induction initiated by the medication cyclophosphamide [122]. Recent research has shown that EMT and CSC have certain commonalities, and their involvements in drug resistance reflect various expressions of the same phenotype; however, the mechanisms of EMT-induced drug resistance are still not well understood. The Wnt, Notch, and Hedgehog signaling pathways are all shared by EMT cells and cancer stem cells (CSCs), suggesting a common mechanism [123]. In this way, EMT provides tumor cells a means to develop resistance to anticancer treatments and avoid the cell death that is normally produced by these medications. Among the best-studied important cytokines in EMT is transforming growth factor beta, whose signaling pathways are linked to acquired drug resistance [124, 125]. Reversing EMT and dramatically increasing cancer cells' susceptibility to chemotherapies have both been linked to TGF- inhibition [126, 127]. Drug resistance has also been linked to the Wnt and Hedgehog pathways, according to the literature [128, 129].

In addition, there is mounting evidence that the EMT program is a crucial regulator of CSCs in mediating drug resistance. Epigenetic alterations triggered by EMT are necessary for cancer cells to enter the CSC state. The field of anticancer therapies would benefit greatly from research into the molecular relationship between EMT, CSCs, and drug resistance [130].

Transcription factors that induce EMT (EMT-TFs) also contribute to the development of drug resistance. Drug resistance may be induced by the overexpression of EMT-TFs [131–135], which include Twist, Snail, Slug, ZEB, and FOXC2. Recent research has shown that mice with pancreatic ductal adenocarcinoma treated with gemcitabine are more sensitive to the treatment and have a higher survival rate when EMT is suppressed by knocking down EMT transcription factors Twist1 or Snail1 [136]. By facilitating

ABC transporter-mediated drug efflux, several of these EMT-TFs contribute to resistance. Binding sites for EMT-TF were identified in the promoters of genes encoding ABC transporters [137]. Through increasing ABCB1 expression and activity, overexpression of Twist, ZEB1/2, Slug, and Snail causes drug resistance [138–140]. It is known that Snail, MSX2, SOX2, and ZEB1 control ABCG2, another ABC transporter intimately associated with MDR [141–144]. EMT-TFs have also been demonstrated to regulate other MDR-related ABC transporters, including ABCC1, ABCC2, ABCC4, and ABCC5. When it comes to paclitaxel-resistant nasopharyngeal cancer cells, for instance, overexpression of ABCC5 is linked to FOXM1. Depletion of FOXM1 or ABCC5 promotes paclitaxel-induced cell death and lowers drug efflux [145–148]. By inhibiting ABC transporters, knocking down these EMT-TFs makes cancer cells more sensitive to chemotherapeutic drugs [142, 143, 148]. Inhibiting metastasis and medication resistance may both be possible by targeting these EMT-TFs.

It is now thought that microRNAs (miRNAs), in addition to EMT-TFs, are critical molecules that connect EMT and ABC transporters [149]. MicroRNAs (miRNAs) are a kind of endogenous RNA consisting of 20–24 nucleotides that may influence the expression of EMT-related genes that belong to the ABC family [149]. The modulation of ABC transporters by microRNAs has been summarized by Haenisch *et al.* [150]. The majority of miRNAs control ABC transporters at post-transcriptional levels by binding sites in the 3′-untranslated region (3′-UTR), although some miRNAs can regulate ABC transporters at transcriptional levels by binding to the gene promoter region [151]. It has been shown that microRNAs (miRNAs) may control not only the expression of ABC transporters but also EMT indicators. MiR-200c, for instance, has been shown to preserve the epithelial phenotype [154] and to negatively regulate EMT by directly targeting 3'-UTR regions of ZEB1 and ZEB2 [152, 153]. Although miR-200c and miR-145 are examples of miRNAs that negatively control ABC transporters and so limit EMT [155], miR-27a is an example of a miRNA that promotes EMT by positively regulating ABC transporters.

An *et al.* outlined the many ways in which miRNAs govern drug resistance, including their roles in modifying apoptosis and autophagy, controlling the metabolism of anti-cancer drugs, affecting therapeutic targets, and influencing DNA repair [156–158]. MiR-134, miR-487b, and miR-655 overexpression increase TGF-induced EMT and treatment resistance to gefitinib in non-small cell lung cancer, according to a recent study [159]. Resistance to EGFR-TKI is induced by this miRNA cluster, which works by directly suppressing MAGI 2, which in turn dampens PTEN function. Acquired resistance to EGFR-TKIs is linked to a loss of PTEN expression

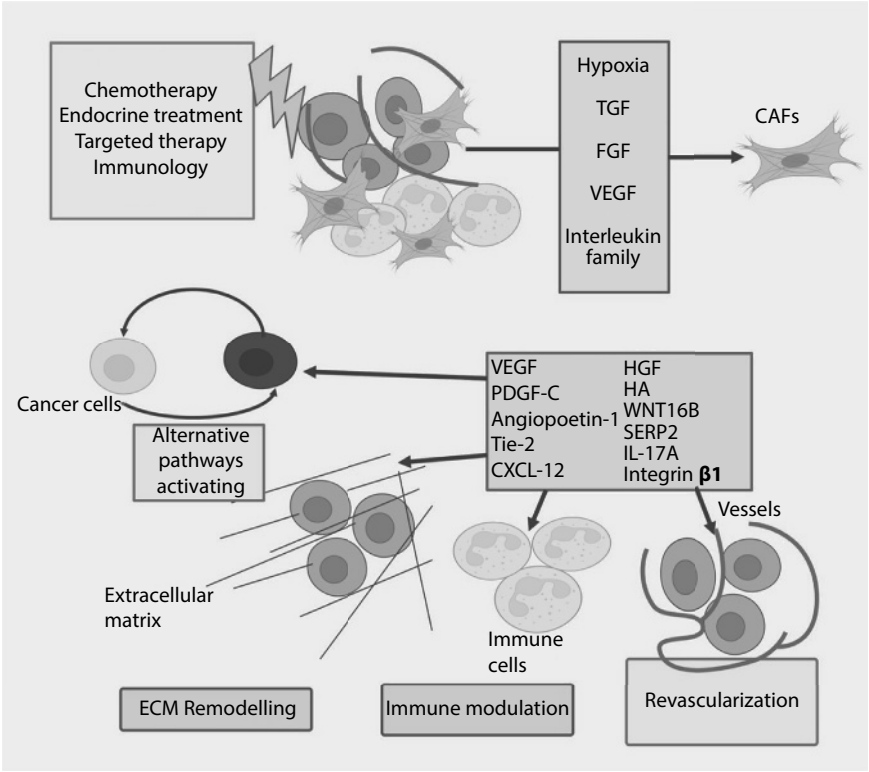

Figure 1.2 Diagrammatic representation of the heterogenous origin of CAFs and their markers.

and an increase in the PI3K–Akt pathway [160]. Heterogeneous origin for CAFs and their marker is shown in Figure 1.2.

1.9 Conclusion

It will be difficult to determine the most effective technique for overcoming medication resistance because of the wide variety of patient malignancies and the intricate nature of tumor growth. High throughput investigations in the fields of cancer genomes, cancer proteomics, and cancer metabolomics have made it feasible to pinpoint the genes and molecules responsible for treatment resistance at any given time during carcinogenesis. Combinational and individualized therapy is necessary because of the heterogeneity of cancer-causing gene alterations. Tumors are often multiclonal

and genetically diverse, making combinational therapy the preferred treatment option. Drug resistance is a major cause of treatment failure when utilizing monotherapy for cancer since the medication kills sensitive cancer cells while allowing resistant cancer cells to survive and grow. However, treatment with two or more medications at once is more likely to suppress numerous clones within a tumor and make it harder for new cancer mutations resistant to multi-drug treatment to be chosen and proliferate.

These driver genes are responsible for the drug resistance processes at work in cancer patients, and current tactics for dealing with drug resistance rely on constant patient monitoring and therapy with a mix of chemotherapeutic/target medicines. Recent advances in targeted drug treatment have shown that targeting many pathways at once may improve therapeutic effectiveness, reduce side effects, and even extend life expectancy for cancer patients. However, the composition/unique resistance profile of tumors and the toxicity tolerance of people make it difficult to anticipate the effects of therapy. Fighting medication resistance may be a never-ending struggle, since tumor cells may constantly find new methods to evade treatment.

Before this, patients with cancer were given the maximum dose of chemotherapy or targeted medications. It has been clear in recent years that this approach of therapy may hasten the development of drug resistance because it constantly stresses tumors, causing them to choose the cancer cells that are most resistant to the medications. Intermittent or adaptive dosing may halt the growth of drug-dependent resistant cells and allow for the competition of sensitive and resistant cells, which is why these novel treatment strategies of "on and off" or "high dose followed by low dose" resulted in longer survival and delayed drug resistance. One study demonstrated that melanoma cells that had developed resistance to combination BRAF- and MEK-targeted therapy exhibited classic signs of drug addiction and were highly vulnerable to sudden discontinuation of treatment. The development of drug dependence has been seen in other cancer types as well, such as lymphoma cells exposed to an ALK kinase inhibitor, indicating that intermittent dosing may increase the duration of time in which ALK+ tumors are under control. These results have bolstered the case for clinical trials of a pulsatile dosing strategy.

While some patients saw an increase in survival because of the adoption of novel therapeutic approaches, others saw no improvement or even worse results. These show that the new approaches can only be employed in certain situations and are not applicable as a whole. More research on drug–cancer interactions at the individual level is necessary before tailor-made treatment strategies can be created.

Isolating the source of the tumor's energy might be one way to get around the resistance they have developed. Even if a tumor is able to avoid a certain pathway, it will still require energy to maintain its growth, proliferation, treatment resistance, and cell migration. Therapeutic reagent effectiveness may be enhanced by the use of energy-blocking combinations. Cancer cells, in contrast to normal cells, do not seem to be as adaptable when it comes to utilizing energy molecules. For instance, it is widely established from positron emission tomography (PET) scans that cancers with a poor prognosis are virtually usually tumors with an active glucose/energy metabolism. Glucose is the preferred source of energy and carbon for certain cancer cells. These malignant cells are "addicted" to glucose and are more sensitive to changes in glucose concentration than normal cells, dying at a much quicker rate in the absence of glucose. A combined therapy strategy using a glucose transport inhibitor or a glycolysis inhibitor and another target medicine may be especially useful in triggering cancer cell death in certain specific cancers.

Recent studies on cancer have shown crucial functions for TME in carcinogenesis and treatment resistance. TME–tumor interactions must be taken into account by any novel medicines that aim to considerably enhance therapeutic results. It has become clear that intratumoral extracellular ATP is one of the TME molecules that has significant effects on tumor cells in terms of proliferation, survival, treatment resistance, and even metastasis. While many other molecules may be employed to catalyze the synthesis of ATP, ATP is the final energy molecule utilized by all cells. Increased ATP levels may be essential for the survival and treatment resistance of cancer cells. Understanding this distinction between cancer and healthy cells may aid in the fight against the disease. Deficiencies in ATP might cause tumors to cease growing or even die if a mechanism is discovered and employed to administer an ATP synthesis inhibitor or extracellular ATP degrader, resulting in reduced ATP internalization within tumors. The anticancer efficiency of both TKIs and chemo medicines may be improved by using them in combination treatment, whereby either extracellular ATP destruction or the prevention of extracellular ATP internalization is considered. Since immune cells in tumors are responsive to ATP levels, it is possible that modifying extracellular ATP levels might further improve cancer immunotherapy.

Finally, the heterogeneity of tumor cells should be lower, drug resistance should be lower, and treatment should be more effective if the tumor is found sooner. The relevance of cancer therapy at later stages should not be underestimated, but neither should the importance of early identification and prevention.

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