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Cancer Stem Cells in Recurrent and Drug-Resistant Lung Cancers

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Abstract:

With a 5-year survival rate of less than 20%, lung cancer is a leading cause of cancer-related deaths worldwide**.** Considering the treatments currently in place, this statistic is frankly shocking. A possible explanation for the disconnect between sophisticated treatments and the survival rate can be found in the Cancer Stem Cell (CSC) hypothesis. The CSC hypothesis suggests the idea of a subpopulation of tumor cells with the abilities of self-renewal, cancer initiation, and further maintenance of tumors**.** Lung CSCs have been associated with resistance to radiation and chemotherapeutic treatments**.** CSCs have also been implicated in recurrent cancers; if the CSCs are not completely killed off after treatment, the cancer tends to reemerge. Extensive investigation of CSCs to determine their responsibility in recurrent and drug-resistant cancers heavily relied on the use of specific markers present in CSCs, including CD133, ALDH, ABCG2, and Nanog**.** Yet another method that results in increased resistance to treatment is epithelial mesenchymal transition, or EMT. Through this process, epithelial cells lose the epithelial phenotype and gain mesenchymal properties. One of these properties is increased drug-resistance, rendering EMT culpable – at least in part – for drug-resistance in cancer cells **.** Furthermore, since miRNA-based therapies are coming to light, various miRNAs will be discussed in terms of their relationship to chemoresistance as well as CSCs in general. Finally, a discussion of the natural and synthetic anti-cancer compounds curcumin, CDF, and BR-DIM will ensue.

1. Introduction:

Lung cancer has two main divisions. These are small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Cases of NSCLC make up 75-80% of all lung cancer cases **(1).** NSCLC can be further subdivided into squamous cell carcinoma, adenocarcinoma, and large cell carcinoma. Most originating sites of the various types of lung cancer are located at or near identified airway stem cell niches, which suggests their stem cell origin **(2)**. The discovery of a group of cells with self-renewing abilities in tumors was groundbreaking, and prompted further study. These cells, called cancer stem cells (CSCs) may be the primary cause of chemotherapy resistance and recurrence in patients with multiple types of cancer, including lung cancer **(3)**. In order to gain a better understanding of lung CSCs and develop new treatments, a fundamental understanding of CSC markers, their therapeutic targeting, drug-resistant properties of CSCs, and the role of miRNAs must be attained. With current understanding combined with further research, we will hopefully continue to develop better and better treatments that can target CSCs as well as removing the bulk of tumors, leading to smaller proportions of recurrence and drug-resistance in lung cancer.

2. Cancer Stem Cells of the Lungs

2.1 Markers defining lung CSCs and their potential implications

In order to research CSCs, they must first be identified. Among the common markers used to isolate and study CSCs are CD133, ALDH, ABCG2, and Nanog. These markers provide ways to assess how effective different treatments are at eradicating CSCs. The markers also have prognostic applications. Furthermore, the markers have been specifically targeted to reduce functionality – or even induce apoptosis – in CSCs, increasing the specificity and thus improving treatment.

2.1.1 CD133

CD133 is a commonly demonstrated lung CSC marker **(2).** It is a cell surface glycoprotein that consists of five transmembrane domains and two large glycosylated extracellular loops **(4).** Researchers tested ten NSCLC cell lines in an attempt to verify that cells positive for CD133 possessed properties of CSCs. Findings suggested that CD133 positive ($CD133^+$) cells showed significantly higher abilities of self-renewal, tumor initiation, and drug resistance when compared to $CD133^-$ cells **(5, 6)**. In addition to these findings in NSCLC cell lines, $CD133^+$ cells with similar CSC properties were found in SCLC, suggesting that CD133 may be a pan-lung cancer stem cell marker **(2)**.

In terms of the potential implications of this marker, high CD133 expression has been linked to poor prognosis in patients with NSCLC **(7, 8)**. This may result from the fact that CD133 expression has also been associated with higher tumor stage in adenocarcinoma **(7)**. However, these results are not completely conclusive yet and cannot be generalized for all patients with NSCLC, as indicated by one study that did not find any link between CD133 expression and NSCLC prognosis **(9)**.

2.1.2 ALDH

Another marker useful for identifying and isolating CSCs has to do with the high aldehyde dehydrogenase activity of stem cells. Aldehyde dehydrogenase (ALDH)'s enzymes control the differentiation of normal stem cells, suggesting a link between ALDH and CSC differentiation **(10).** Furthermore, the ALDH family of intracellular enzymes was found to participate in cellular detoxification and drug resistance in CSCs **(2).** ALDH1, a cytosolic isoenzyme, is a member of the ALDH family. Lung cancer cells that expressed ALDH1 demonstrated highly tumorigenic and clonogenic properties. **(11)**. Moreover, $ALDH1A1^+$ CSCs

displayed resistance to chemotherapy drugs and EGFR-TKI (epidermal growth factor receptor tyrosine kinase inhibitors), both treatments used to fight lung cancer **(12)**. Specifically, the drugs to which ALDH1A1^{+} CSCs displayed resistance are the chemotherapeutic drugs cisplatin, etipisode, and fluorouracil, as well as the EGFR-TKI gefitinib **(12)**.

Taking a look at the combined effects of the overexpression of CD133 in conjunction with that of ALDH, it is important to note that this combination has been related to an increased risk of recurrence in early-stage NSCLC **(13).** Furthermore, the concomitant expression of CD133 and ALDH1A1 was correlated with shortest overall survival among 205 stage-1 NSCLC patients **(13)**. Thus, the detection of both CD133 and ALDH could potentially serve as a prognosis indicator for NSCLC patients.

2.1.3 ABCG2

Yet another marker of lung CSCs is ABCG2, an ATP-binding cassette transporter. ABCG2 has the ability to pump chemotherapeutic drugs outside the cell, ultimately resulting in decreased intracellular concentrations of the drugs **(4).** The transporter works by using energy from ATP to drive the active transport of drug metabolites and other compounds across the cell membrane. The ATP-binding cassette (ABC) superfamily, of which ABCG2 is a part, is a powerful resistance mechanism that greatly contributes to the chemoresistance of CSCs **(14, 15)**. Looking at the implications of the presence of the ABCG2 marker, the source of the energy driving its active transport becomes important. Since ABC transporters are ATP-dependent, ATP-competitive agents could target them to potentially reduce their efficacy.

2.1.4 Nanog

The Nanog transcription factor plays a key role in maintaining the self-renewal of embryonic stem cells in embryonic development **(16, 17)**. It plays a similar role in CSCs. Since

its role is directly related to such a key phenotypic characteristic of CSCs, it has been used as a marker in lung CSCs **(17, 18)**. The overexpression of the Nanog protein predicted a worse prognosis for lung cancer patients, suggesting its possible use as a prognosis indicator **(19)**. The relationship between Nanog and lung CSCs needs to be further examined in order to continue the development of novel treatments.

2.2 Therapeutic targeting of lung CSC markers

Therapeutic treatments have been developed in attempts to specifically attack CSCs. Such treatments have made use of CSC markers by either using them to find CSCs or by actually targeting the markers themselves. The therapeutic targeting of lung CSC markers has not been studied to the depth it merits in lung cancer. However, markers of lung CSCs have indeed been established and studied in great detail. Thus, this section will entail the discussion of therapeutic targeting of lung CSC markers in any type of cancer._Some lung CSC markers that have been targeted include the aforementioned CD133, ALDH, ABCG2, and Nanog.

CD133 targeting in human metastatic melanoma has been effective. Short hairpin RNAs were used to down-regulate CD133. This led to decreased movement ability, spheroid-forming ability, and capacity of metastasis **(20)**. The down-regulation also led to slower overall cell growth. An efficient method in the elimination of $CD133^{+}$ tumors is the use of antibody-drug conjugates **(21)**. This method has been used with success in hepatocellular and gastric cancers, and its efficiency when applied to lung cancer should be further studied.

Yet another marker targeted in lung cancer is the ALDH family. The ALDH family has been targeted in colorectal cancer and breast cancer, among others. In both colorectal and breast cancer, ALDH1 activity inhibition with DEAB was successful. In breast cancer cells, the ALDH1 inhibition resulted in suppression of tumor-initiating ability and a reduction of

metastasis to the lungs **(22)**. In colorectal cancer cells, ALDH1 inhibition caused treated lines to be more sensitized to the cytotoxic effects of a chemotherapeutic drug, CPA **(16)**. Another method used in colorectal cancer cells was the down-regulation of ALDH through the use of shRNA, which reduced the number of detected CSCs **(16)**.

As previously discussed, the ABC multidrug efflux pumps are important for the chemoresistance of CSCs. In order to increase the potency of treatment, the ABCG2 transporter has been targeted. Inhibitors of the transporter are still waiting comprehensive clinical assessment, but they include phosphodiesterase-5 inhibitors and Ko143 **(23)**. Dietary flavonoids may also work to inhibit ABCG2 –mediated cellular drug efflux **(24)**. Such inhibitors will hopefully eventually work synergistically with conventional chemotherapeutics to eliminate tumors and reduce possibilities of recurrence in cancers.

Finally, Nanog mRNA knock-down has resulted in decreased mobility and invasion abilities of choriocarcinoma cells **(25)**. Since the therapeutic targeting of Nanog has proven successful in one type of cancer, it has the potential to be successful in the treatment of lung cancer as well.

3. Drug-resistance

Therapeutic resistance is one of the primary causes of failure in cancer treatment **(26)**. Drug-resistant properties of cancer can result in either an immediate re-initiation of the disease or a re-initiation after a significant lapse of time **(27)**. Some common treatments of lung cancer that have faced the problem of treatment resistance include Epidermal Growth Factor Tyrosine Kinase Inhibitors (EGFR-TKI), chemotherapy, anti-proliferative treatments, and radiation treatment **(27-29).** The process of EMT has played a role in drug-resistance, as have the very properties of CSCs.

3.1 Role of EMT in drug-resistance

Epithelial cells can become invasive, migratory mesenchymal cells. This process, known as epithelial mesenchymal transition (EMT) gives cancer cells the ability to migrate, invade, and spread through the blood. Furthermore, EMT may result in the production of CSCs, as evidenced by differences in cell surface marker expression and increased tumor formation **(30-32).** Typical progression of EMT involves losing epithelial markers and gaining mesenchymal markers **(33)**. A distinctive feature of EMT is the loss of E-cadherin, a glycoprotein involved in epithelial cell-cell adhesion and cytoskeletal organization **(26)**. Considering its primary functions, it is clear that E-cadherin would not be useful for a migratory mesenchymal cell.

The loss of function of E-cadherin is thought to enable metastasis by giving rise to significant transcriptional and functional changes. One particular study focused on the role of E-cadherin in EMT. This study sought to determine whether E-cadherin loss resulted solely in the loss of cell-cell contacts or if E-cadherin loss activated multiple transcriptional pathways. Results indicated that E-cadherin loss contributed to the action of multiple transcriptional pathways **(26, 34)**. In fact, after E-cadherin loss, 19 transcription factors were highly induced. Moreover, E-cadherin loss alone is enough to give metastatic abilities to breast cancer cells that previously lacked these abilities **(35).**

EMT plays a key role in making cancer cells drug-resistant to commonly used therapeutics, such as EGFR-TKI. EGFR is an oncogenic pathway that has been inhibited through the use of tyrosine kinase inhibitors (TKIs) **(29)**. EGFR-TKI has been used to treat the adenocarcinoma subset of NSCLC **(29)**. Though patients respond to the treatment initially, most patients face relapse **(36)**. Adenocarcinoma cells resistant to EGFR inhibitors such as gefitinib and erlotinib showed a decrease in their expressions of E-cadherin, an epithelial cell marker, and

an increase in their expressions of vimentin, a mesenchymal cell marker. Since the drug-resistant lung cancer cells display the mesenchymal phenotype, EMT might be an indicator of insensitivity to EGFR inhibition in lung cancer **(26)**. Furthermore, restoration of E-cadherin increased the sensitivity of the drug-resistant cancer cells to EGFR-TKIs such as gefitinib, further suggesting a relationship between EMT and resistance to EGFR-inhibitors **(29)**. Though support for the relationship between EMT and resistance to these inhibitors in adenocarcinoma is present, the evidence is still inconclusive. For example, one particular study found that only 50% of samples had undergone EMT after exposure to gefitinib **(37)**. Further research is required to fully understand the relationship between EGFR-TKI resistance and EMT. Such research may help increase the efficacy of EGFR-TKI in patients who have shown resistance to this treatment method.

3.2 Role of CSCs in drug-resistance

Some therapies that are currently in place are effective in that they are able to remove bulky disease. However, therapies that fail to employ a strategic elimination of CSCs are often ineffective and result in cancer recurrence. **(27)**. A specific example of such an instance can be seen in platinum-based combination chemotherapy, a first-line treatment for NSCLC in advanced stages **(28)**. This type of treatment works by inhibiting DNA repair and/or DNA synthesis in cancer cells. Notably, a significant number of patients face tumor recurrence after platinum-based combination chemotherapy **(38)**. When first-line agents fail, second-line agents (such as docetaxel and pemetrexed) are used. Unfortunately, the second-line agents tend to be ineffective in patients who have received typical first-line chemotherapy. A recent study discovered that cisplatin treatment, a platinum-based first-line treatment, elevated the ratio of cells expressing

the CSC markers CD133 and Nanog **(14, 28)**. The cisplatin treatment selected for CSCs, resulting in the high rate of paclitaxel resistance in patients who had been treated with cisplatin.

CSCs have special properties that contribute to their drug-resistance. Some of the more significant contributing properties include CSCs' relative dormancy, their high capacity for DNA repair, and their high expression of multiple drug resistance membrane transporters **(27).**

The relative dormancy of CSCs is important when considering anti-proliferative treatments, such as imatinib and nolitinib **(27)**. CSCs are often in a state of dormancy, or quiescence, where they are non-proliferative **(39)**. While CSCs are not in the cell cycle, they are protected from chemo-radiotherapy. The use of specific cytokines (like As_2O_3) to force the CSCs to re-enter the cell cycle can restore chemo- and radio-sensitivity and should be employed in conjunction with anti-proliferative treatments **(40)**.

CSCs express a significant amount of multiple drug resistance membrane transporters, including those of the ABC family **(27, 41)**. As previously discussed, these transporters use active transport to efflux drugs, reducing the drugs' impact on CSCs **(14)**. However, CSCs rely on still other mechanisms for drug resistance, limiting the efficacy of ABC transporter inhibitors.

Furthermore, CSCs have a high capacity for DNA repair, yet another factor contributing to their drug-resistance **(27)**. In a study of human glioblastomas, $CD133^+$ cells were found to survive radiation treatment better than cells without this CSC marker **(42)**. This survival difference can be attributed to the efficient DNA repair mechanisms present in CSCs, such as the Chk1 and Chk2 checkpoint kinases **(42)**. These kinases pause the cell cycle to allow DNA repair to happen.

4. MiRNAs

MicroRNAs, or miRNAs, are non-coding RNAs made up of 19-22 nucleotides that help regulate gene expression during translation **(43)**. MiRNAs play very important roles in numerous biological processes of cancer cells, including development, proliferation, and apoptosis **(44)**. MiRNAs are endogenous posttranscriptional regulators that negatively regulate expression of their target genes **(45)**. MiRNAs can be either oncogenic or tumor suppressing, depending on the subsequent pathways they influence. MiRNAs will be discussed in terms of their impact on chemoresistance and the maintenance of CSCs in general. New therapies take advantage of knowledge gained from miRNA research, making the understanding of how miRNAs are involved in cancer critical.

4.1 MicroRNAs associated with chemoresistance

Since miRNAs are useful in so many different arenas, it is only natural that they be discussed in relation to chemoresistance. Much research has focused on – and continues to focus on – the up-regulation or down-regulation of miRNAs in relation to treatment resistance. This research can result in attempts to up-regulate the miRNAs to reverse treatment resistance or developments of new treatments altogether. Since miRNAs are associated with chemoresistance, they can also prove useful as prognostic indicators. The miRNA-212, the let-7 family, and various miRNAs associated with EGFR should be further researched in order to identify new treatments or improve the effectiveness of currently common treatments for lung cancer.

4.1.1 miRNA-212

miR-212 is considered a tumor suppressor, and its down-regulation has been correlated with chemoresistance **(46)**. When miR-212's expression levels are normal, it increases tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced cell death in NSCLC cells **(47)**. The down-regulation of miR-212 leads to the up-regulation of the anti-apoptotic PED. PED

has been implicated in inducing resistance to chemotherapeutic treatment, meaning that miR-212 down-regulation is responsible in part for chemoresistance. Another way miR-212 may be involved in chemoresistance can be found by considering its relationship with ABC multidrug efflux transporters. In CML, or chronic myeloid leukemia, miR-212 down-regulation corresponded with decreased ABCG2 protein expression **(48)**. The use of reporter gene assays established that miR-212 targeted the 3'-UTR region of ABCG2 **(48)**. Finally, MiR-212 down-regulation has also been indicated as responsible for docetaxel resistance in NSCLC adenocarcinoma cells **(49).**

4.1.2 The let-7 family

Let-7 (lethal-7) refers to a family that consists of 12 miRNAs. The let-7 family is a known inhibitor of EMT **(50)**. Accordingly, let-7 was down-regulated in A549 NSCLC cells treated with TGF-β1. These cells were resistant to the drug erlotinib. The re-expression of let-7b and let-7c led to an appeared reversal of EMT and was accompanied by increased erlotinib sensitivity **(51).** Since let-7 down-regulation results in drug resistance, it follows as logical that reduced levels of let-7 have been associated with poor patient outcome for patients with lung cancer **(52)**.

4.1.3 miRNAs associated with EGFR

Acquired resistance of NSCLC to EGFR has included secondary mutations in EGFR itself. An example of such a mutation is the EGFR T790M "gatekeeper" mutation, which has been responsible for 50% of resistant cases **(53)**. Another mechanism of acquired resistance, the amplification of the MET oncogene, has been associated with tumor growth and metastasis. This mechanism has been observed in 20% of resistant cases **(54)**. The study of miRNAs through a microRNA microarray identified that miR-30b, miR-30c, miR-221, and miR-222 target both

epidermal growth factor (EGF) and MET receptors **(55).** The microarray also found that miR-103 and miR-203 target solely the MET oncogene. These microRNAs collectively had a large impact on the response to gefitinib-induced apoptosis of NSCLC. The microRNAs inhibited expression of genes encoding BCL2-like 11 (BIM), apoptotic peptidase activating factor 1 (APAF-1), protein kinase C-ε, and sarcoma viral oncogene homolog (SRC) **(56)**. Modulating these miRNAs, in conjunction with chemotherapy, could provide a better outlook for NSCLC patients treated with EGFR-TKIs.

4.2 MicroRNAs associated with CSCs

MiRNAs, important regulators of CSCs, are improperly regulated in many cancers. Such incorrect regulation could include the down-regulation of tumor suppressors, as is seen in miRNA-34a, miRNA-21, and the miRNA-200 family. This process brings to mind the question of whether re-introduction or corrected regulation of these miRNAs would restore normal tumor suppressing ability. Interestingly enough, in some cases, it has.

4.2.1 miRNA-34a

MiR-34a is a known tumor suppressor, and its up-regulation has led to increased apoptosis **(46)**. The tumor suppressor p53 transcriptionally induces miR-34a. **(57)**. When miR-34a expression is reduced, lung CSCs take on a more aggressive phenotype **(58)**. When miR-34a expression is increased again, this more aggressive phenotype is lost **(58)**. Since miR-34a is frequently down-regulated in lung cancer, it is being evaluated as a replacement therapy candidate **(55)**. The delivery of a miR-34a mimic has been shown to reduce tumor growth **(59)**. This suggests that miRNA replacement therapy may prove extremely useful and encourages further research into the field of miRNAs related to CSCs – and cancer in general.

4.2.2 miRNA-21

MiRNA-21 expression was greatly increased in colon cancer CSCs **(45)**. The down-regulation of miR-21 caused the differentiation of CSCs, as evidenced by a decrease of CSC markers. Since differentiated CSCs are more susceptible to treatments, down-regulation of miR-21 in conjunction with other treatment was studied. When the down-regulation of miR-21 preceded other treatments such as FUOX and CDF, the treatments were more effective **(45)**.Taking a more detailed look at miR-21, it is important to consider its targets. Phosphatase and tension homolog (PTEN) is a tumor suppressor gene that is a target of miR-21 **(60).** When miR-21 is suppressed, PTEN is up-regulated, resulting in tumor suppression **(60)**. In relation to lung cancer, miR-21-3p relative expressions were found to be higher in NSCLC tissues as compared to non-cancerous tissues **(61)**. miR-21, however, has a lower prognostic value when compared to other miRNAs, so while down-regulation of miR-21 should be attempted in lung cancer, the expectations for its impact on lung CSCs should not be too high.

4.2.3 The miR-200 family

The miR-200 family is a known inhibitor of EMT, a process that is responsible for some of the production of CSCs **(30-32, 51).** The loss of expression of the miR-200 family is associated with an increase in EMT, and consequently drug resistance and CSCs **(60)**. MiR-200b, a member of the miR-200 family, targets Suz12. The expression of Suz12 is enough to generate CSCs **(62)**. The re-expression of miR-200 through the use of drugs such as CDF would inhibit Suz12, helping to suppress tumor growth and stop the generation of CSCs **(62)**. This re-expression could also potentially reverse EMT, lead to the differentiation of CSCs, and improve prognosis of lung cancer **(63)**.

5. Natural and synthetic anti-cancer compounds

The discovery of anti-cancer compounds, both natural and synthetic, is very interesting in that though we may be searching for compounds with astounding effects on cancer, we are also interested in learning how these compounds function. The increased understanding of both natural and synthetic anti-cancer compounds can result in the discovery or synthesis of novel compounds that may have a profound impact on the face of cancer treatment worldwide. In this section, a natural compound, BR-DIM, will be discussed, as will another natural compound, curcumin, and its synthetic analog, CDF.

5.1 BR-DIM

One treatment that has proven in the elimination of cancer cells is the BR-DIM treatment **(64).** This natural agent works in part by inducing apoptosis in lung cancer cells by the down-regulating Survivin and Bcl-2, decreasing Bax, and enhancing procaspase cleavage **(65, 66)**. This agent also induces apoptosis through activation of the p38 MAPK pathway **(67)**. In NSCLC, BR-DIM was shown to inhibit the growth of drug-resistant cell lines that exhibited mutant EGFR **(66)**. Even cancer cells resistant to targeted therapies, chemotherapy, or radiation exhibited growth inhibition in the presence of BR-DIM **(66)**. Met, which has been linked to poor patient prognosis in lung cancer, faced reduced expression in lung cancer cells when they were treated with BR-DIM **(66)**.

Most significantly, BR-DIM may be able to reduce cancer metastasis or recurrence. Such an outcome is possible due to BR-DIM's ability to decrease invasive abilities of EGFR events. A possible mechanism for this is the suppression of the pro-metastatic chemokine receptor CXCR4 **(68, 69)**. This compound should be studied in combination with other forms of therapy to find treatment that will provide the best prognostic outlook for patients.

5.2 Curcumin

One example of a natural anti-cancer compound is curcumin. Curcumin is a non-toxic substance extracted from turmeric **(43).** Curcumin has proven effective in inducing the apoptosis – as well as inhibiting the proliferation – of drug-resistant CSCs. Some ways in which curcumin are effective include inducing EGFR removal-related apoptosis, increasing CSC treatment sensitivity, and interacting with miRNAs to induce apoptosis **(43)**.

As previously discussed, the EGFR-TKI method of NSCLC treatment is prone to resistance. However, when curcumin is present, the EGFR protein undergoes ubiquitination and degradation **(70)**. Decreasing the EGFR protein on the cell membrane results in eventual cancer cell apoptosis and death **(70)**. Part of what makes this method successful is the fact that it is not susceptible to EGFR mutation.

Furthermore, curcumin may increase the therapeutic effectiveness of existent treatments. Curcumin was able to induce the sensitivity of $CD133^+$ CSCs in laryngeal carcinoma to cisplatin. This resulted in the reduction of the percentage of $CD133^+$ CSCs, which were previously resistant to treatment **(71)**. Curcumin was able to reduce drug-resistant properties by down-regulating the expression and/or activity of ABC multidrug transporters in leukemic cells **(43)**. ABCG2, a member of this family of transporters, is also a marker for lung CSCs, suggesting curcumin's potential efficacy in reducing drug-resistant properties of lung CSCs. Curcumin has also reduced amounts of $CD133^+$ medulloblastoma, glioblastoma, pancreatic and colon CSC proliferation through Hedgehog, insulin growth factor (IGF-), STAT3-, and histone methyltransferase EZH2-dependent mechanisms **(70)**. CD133 is also a marker used for the identification of lung CSCs in both NSCLC and SCLC, again suggesting curcumin's potential effectiveness in lung cancer. Curcumin works to reduce drug-resistant properties of cancer by

targeting CSCs and their markers. This may indirectly result in a reduction of tumor recurrence, since CSCs have been posited as being responsible for the phenomenon **(27, 72).**

Curcumin has also been able to induce apoptosis in a multi-drug resistant lung adenocarcinoma cell line, A549 **(73)**. By down-regulating miR-186 in A549 (lung adenocarcinoma) cells, curcumin was able to promote lung cancer cell apoptosis **(74)**. Discoveries of the efficiency of curcumin in lung cancer treatment (and cancer treatment in general) prompted research into the field of curcumin analogs, some of which have been extremely useful in the continuing fight against cancer.

5.3 CDF

The low bioavailability of curcumin prompted the synthesis of CDF, a difluorinated synthetic analog of curcumin with greater bioavailability **(3, 60).** CDF works in a manner similar to curcumin. It down-regulates the expression and/or activity of EGFR, IGF-1R, NF-κB, c-Myc, β-catenin, COX-2, and the ABCG2 multidrug transporter **(3).** In order to ensure that the efficiency of CDF at killing CSCs was on par with that of curcumin, tests were conducted comparing the two in terms of their ability to reduce the presence of CSC markers in chemo-resistant colon cancer cells that were highly enriched in CSCs. These tests discovered that CDF was more effective.

CDF was determined to cause a greater induction of overall apoptosis **(43)**. CDF promoted this apoptosis by activating the pro-apoptotic factor Bax **(3)**. Furthermore, CDF was able to inhibit and disintegrate colonospheres containing over 80% of CSCs, as determined by presence of the colon CSC marker CD44 **(3)**. Curcumin failed to do the same. In a study comparing CDF and curcumin in the pancreatic cancer cell lines AsPc-1 and MIAPa-Ca-2, similar results were observed **(60)**. In order to determine which is superior in terms of killing

lung CSCs, further research needs to be conducted. Furthermore, the specificities of the mechanisms of CDF have not yet been determined in their entirety.

6. Conclusions – The Future of CSCs and CSC-targeted Treatment

Research on CSCs has shed enormous light on why so many cancers are drug-resistant and recurrent. With this powerful information, treatments can be modified to include components that kill CSCs as well as the regular mass of cells. Understanding the processes of how CSCs confer drug-resistance can lead to treatment with the goal of eliminating drug-resistant properties before – or while – administering traditional treatment.

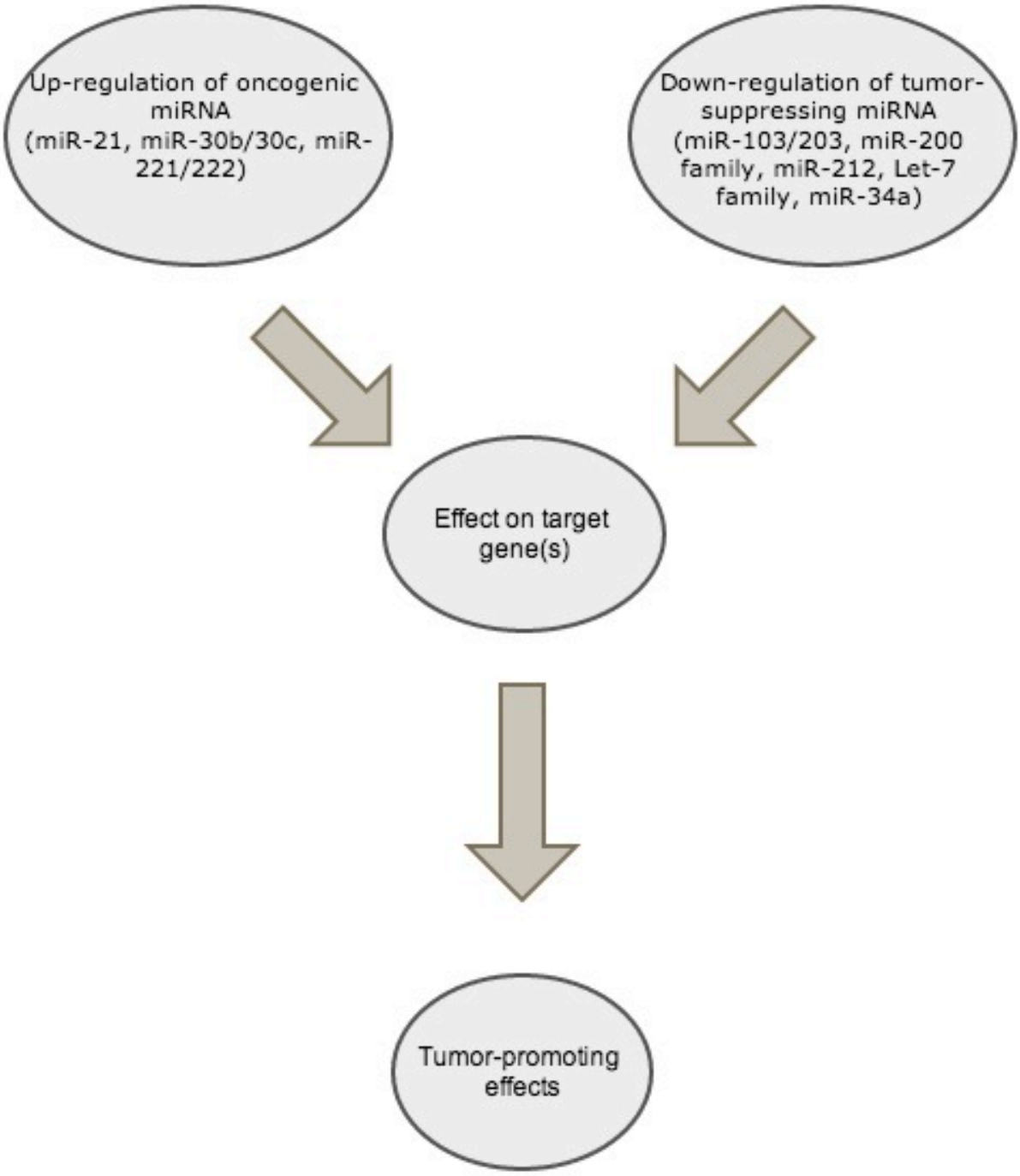
CSCs have already changed the face of cancer treatment. Already, the use of antibody-drug conjugates that target markers of CSCs in addition to normal drug function have been successfully employed **(21)**. Such ideas are delightfully simple, but they could not have ever come into existence without countless hours in the laboratory determining the very existence of CSCs, pinpointing their markers, finding an antibody, and creating the antibody-drug conjugate. The antibody-drug conjugate is just one example of a success story.

Novel natural and synthetic compounds that target CSCs are slowly being understood. These compounds can help us as human beings make lifestyle choices and changes, where possible, to reduce risk of cancer. Prevention is always more efficient than treatment. For example, BR-DIM and curcumin can be added to the standard diet fairly easily **(7)**. Further research into these compounds may result in the eventual synthesis of a compound superior to all existent compounds. The possibilities are endless.

CSCs are – and should be – at the forefront of cancer research. The practical applications surrounding their research are absolutely astounding. Future research should focus on new ways to target CSC markers, methods to induce CSC differentiation to reduce drug-resistance, the use

of miRNAs in treatment, and, again, heightened understanding of anti-cancer compounds. By continuing to amalgamate more knowledge, there is hope for improved prognosis for patients afflicted with cancer of all types.

MiRNA	Up or Down-regulated in Cancer	Target Genes	Reference Numbers
MiR-21	Up-regulated	PTEN	45, 60, 61
MiR-30b/30c	Up-regulated	BIM/APAF-1	46, 55, 56
MiR-221/222	Up-regulated	BIM/APAF-1	55, 56, 63
MiR-103/203	Down-regulated	PKC-ε, SRC	55, 56
MiR-200 family	Down-regulated	E2F3	55, 60, 62
MiR-212	Down-regulated	PED	46, 47, 48, 49
Let-7 family	Down-regulated	RAS, HMGA2	50, 51, 52
MiR-34a	Down-regulated	c-Met, CDK4, Bcl-2	46, 55, 57, 58, 59


Up-regulation of oncogenic miRNA (miR-21, miR-30b/30c, miR-221/222)
Down-regulation of tumor-suppressing miRNA (miR-103/203, miR-200 family, miR-212, Let-7 family, miR-34a)
Effect on target gene(s)
Tumor-promoting effects

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