The scope of the book encompasses topics with a latitude from adult stem cells to pluripotent stem cells in regenerative medicine either alone or as part of the combinatorial therapeutic intervention approach as "drug" besides their application as tools during drug development process. Written by a leading group of researchers, the book encompasses experimental to clinical aspects of stem/progenitors cells, their biology, and characteristics.

- Covers a wide range of topics from adult stem cells to pluripotent stem cells
- Describes stem cells as drugs (therapeutic interventions) and as tools (disease model)
- First-hand research findings written by internationally renowned experts

Prof Dr Khawaja Husnain Haider
is Professor and Chair, Sulaiman Al-Rajhi Medical School, Kingdom of Saudi Arabia having multiple academic, teaching and research affiliations around the globe. He has authored more than 250 research papers, review articles, abstracts and book chapters and served as investigator on multiple NIH-funded stem cell research projects.

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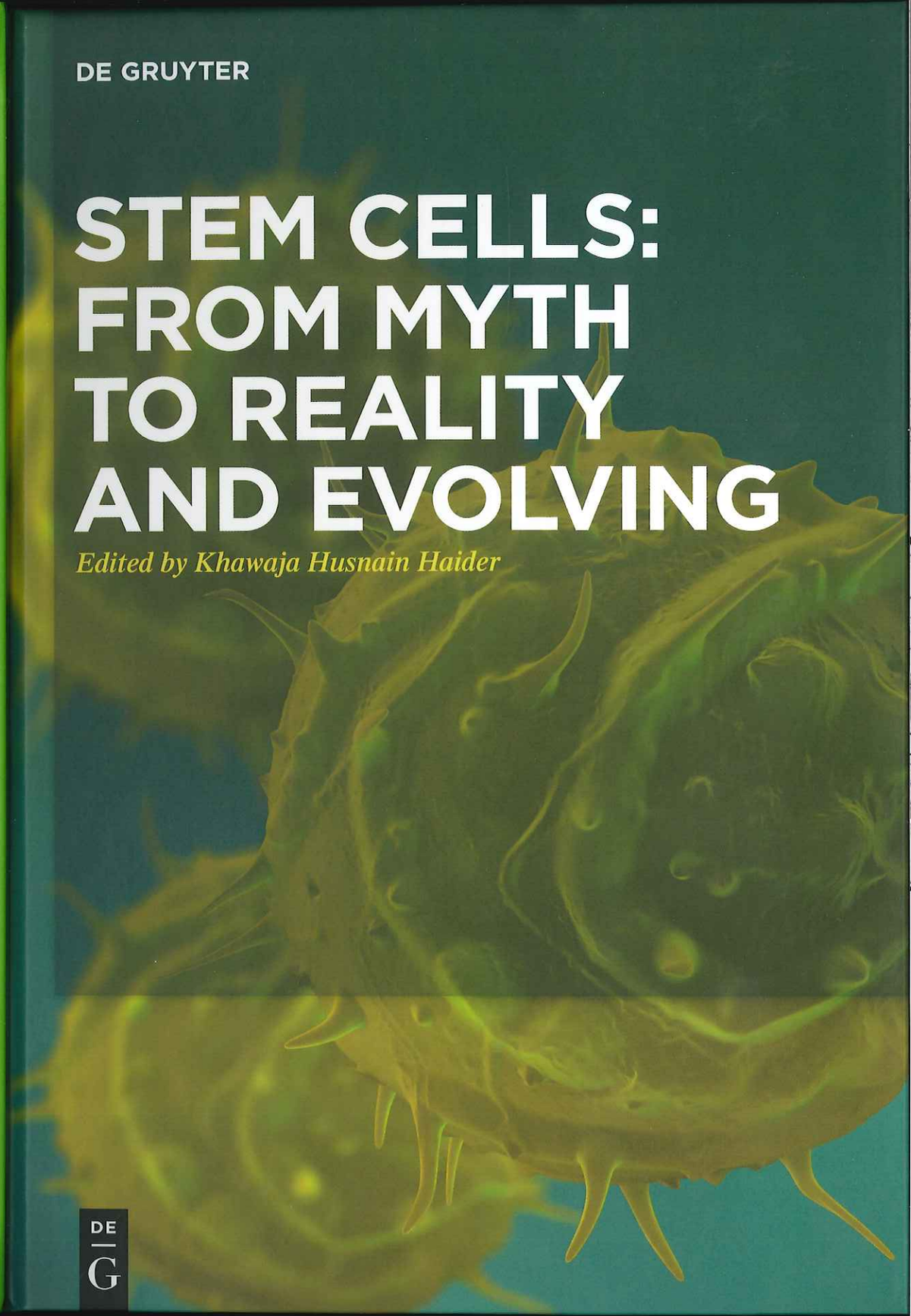

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Khawaja Husnain Haider (Ed.)

Stem Cells – From Myth to Reality and Evolving

DE GRUYTER

Contents

Valentina Masciale, Giulia Grisendi, Uliano Morandi,
Massimo Dominici and Beatrice Aramini

2 Stem cells and lung cancer: between advanced diagnostics and new therapeutics

Abstract: Lung cancers (LCs) remain a significant and devastating cause of morbidity and mortality worldwide. Despite the very recent success of immunotherapy, the diagnosis and treatment of LC remain one of the greatest challenges in chest surgery, clinical oncology, and molecular medicine. A growing number of investigations on normal/cancer stem cells and cellular therapies are offering exciting new avenues to advance knowledge on LC. Here, we will be focusing on the multiple relationships between LC and stem cells accounting for cancer stem cell (CSC) diagnostics and progenitor-based therapeutics for LC. Cancer cell repopulation after chemotherapy and/or radiotherapy still represents a major factor limiting the efficacy of treatment since CSCs play critical roles during this process by reciprocal connections between CSCs and tumor microenvironment. This calls for new opportunities to integrate advanced CSC diagnostics and targeted approaches also based on immunotherapy. In addition, recent discoveries on malignant pleural and other LC highlight that mesenchymal stromal/stem cells may be a novel platform for drug delivery within still unexplored gene therapy strategies. This chapter will dissect these two apparently distant technologies within a unified stem-cell-based vision aimed at providing better diagnostics and therapeutics for LC at the forefront of modern clinical oncology.

Key Words: Cancer, Carcinoma, Lung, MSCs, Stem cells.

2.1 Introduction

2.1.1 Lung cancer epidemiology

Lung cancer (LC), which has a low survival rate, is a leading cause of cancer-associated mortality worldwide. Reports of LC in the scientific literature date back to the early 1400s, when up to 50% of miners working along the border of Germany and the Czech Republic died of a pulmonary disease called *bergkrankheit* (mountain disease) [1–3]. In 1879, Harting and Hesse performed 20 autopsies on miners and described pulmonary sarcoma in 75% of these patients diagnosed with *bergkrankheit*. It was hypothesized that dust inhalation was a causative factor of this illness, which was later identified as squamous cell carcinoma of the lung [4]. Investigators in the 1920s and 1930s proposed radiation and radon gas as potential etiologic agents. With the incidence

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of LC increasing in the 1930s, Ochsnerand De Bakey reviewed the increasing number of LCs among their patients and concluded that cigarette smoke inhalation was a probable responsible factor [5]. Sir Richard Doll and Austin Hill's landmark article in 1950 described mounting evidence that LC was associated with cigarette smoking [6]. The 1962 report by the Royal College of Physicians and the 1964 warning by the surgeon general of the United States firmly established the correlation between cigarette smoking and LC [6, 7]. It is now known that most deaths from LC (up to 85%), which is now the leading cause of cancer mortality in the United States, are directly attributable to smoking [8, 9].

Incidence and mortality attributed to LC have risen steadily since the 1930s, predominantly due to the popularity of cigarette smoking [1]. In the past 100 years, LC has therefore been transformed from a rare disease into a global problem [1].

In 2012, the world age-adjusted incidence rate of LC was 34.2/100,000 for men and 13.6/100,000 for women. The rate translated to 1.82 million new LC cases (1.24 million men and 0.58 million women), an increase from 2002 estimates (1.35 million for both genders) [10]. Among the geographic regions, males in Central and Eastern Europe had the highest incidence rate (53.5/100,000), followed by males in Eastern Asia (50.4/100,000). The highest incidence rates among females were in North America (33.8/100,000) and Northern Europe (23.7/100,000).

With regard to mortality, in 2012, the world age-adjusted mortality rate of LC was 30.0/100,000 for men and 11.1/100,000 for women. There were 1.59 million deaths attributable to LC, and the number has increased from 1.18 million deaths in 2002 [10]. Among the geographic regions, males in Central and Eastern Europe had the highest mortality rate (47.6/100,000), followed by males in Eastern Asia (44.8/100,000). The highest mortality rates among females were in North America (23.5/100,000) and Northern Europe (19.0/100,000). Smoking and air pollution are the major causes of LC; however, numerous studies have demonstrated that genetic factors also contribute to the development of LC. Despite very recent success of immunotherapy, the diagnosis and treatment of LC remain one of the greatest challenges in chest surgery, clinical oncology and molecular medicine.

In fact, the low survival rate in LC patients is related to the stage of LC at diagnosis. In the United States during 2005–2011, LC patients diagnosed when localized and regional had moderate 5-year survival rates (55% and 27%, respectively); however, this decreased to 4% for those with distant cancer [11]. Due to the nonspecific nature of LC symptoms, the majority of LCs are typically diagnosed after it has advanced (57% of LCs in the United States are detected with metastases) [11].

2.1.2 LC *standard* treatments

Surgery is the cornerstone of management for patients with early-stage (I–II) non-small cell lung cancer (NSCLC) and selected patients with stage IIIA disease (T3N1)

according to TNM (T= Primary Tumor; N= involvement of lymph node; M= metastasis) 8th edition [12, 13]. The Lung Cancer Study Group concluded that lobectomy is a superior operation for T1N0 NSCLC, based on a randomized trial of lobectomy versus more limited resection [14]. Thus, in patients with stage I and II NSCLC who are medically fit for conventional surgical resection, lobectomy or greater resection is recommended rather than sublobar resections (wedge or segmentectomy) [12]. In patients who, for medical reasons (severely compromised pulmonary function, advanced age, or other extensive comorbidity), cannot tolerate a full lobectomy, a more limited operation (sublobar) is recommended [10]. For patients with more advanced tumor in whom complete cancer resection cannot be achieved with lobectomy, sleeve lobectomy is recommended over pneumonectomy because it preserves pulmonary function [12].

Although surgery is the treatment of choice for NSCLC patients with early-stage disease, some never undergo surgery. Common reasons for not undergoing surgery are older age, the presence of serious comorbidities, and patient refusal. For those patients who do not undergo an operation, radiotherapy can be administered with curative intent, albeit with lower survival rates when compared to surgery [15, 16].

Recent data from randomized adjuvant clinical trials [17–19] and a recent meta-analysis [20] have changed the standard of care for patients with completely resected NSCLC. The survival benefit observed with adjuvant chemotherapy was confirmed by a meta-analysis of five randomized trials [17, 18, 20–22] with 4584 patients registered in the Lung Adjuvant Cisplatin Evaluation database [19]. This meta-analysis demonstrated a 5.4% increase in 5-year survival in favor of adjuvant chemotherapy compared with observation (hazard ratio: 0.89; 95% confidence interval: 0.82–0.96) [20]. The survival benefit varied according to stage and was most pronounced for patients with stage II and IIIA disease. The improvement in survival in patients with stage IB disease did not reach statistical significance. Patients with stage IA disease appeared to do worse with adjuvant chemotherapy. Some retrospective data suggest that patients with stage IB disease and tumor ≥4 cm may also benefit from adjuvant chemotherapy [23]. With regard to postoperative radiotherapy (PORT) meta-analysis [24, 25], which included 2128 patients, it has been demonstrated that the use of PORT was associated with a detrimental effect on survival, which was more pronounced for patients with lower nodal status. This analysis has been criticized, however, for its long enrolment period and use of different types of machines, techniques, and doses.

Up to one-third of patients with NSCLC present with disease that remains local to the thorax but is considered too extensive for surgical treatment (stages IIIA and IIIB). Concurrent chemoradiotherapy is considered the standard therapy for unresectable stage III NSCLC [26]. The concurrent administration of chemotherapy plus radiotherapy results in a modest but statistically significant survival benefit compared with sequential administration, as demonstrated by randomized phase III trials

[27, 28]. This approach is, however, associated with significant toxicity and it applies only to patients with good performance status [29].

There is another issue influencing the battle against cancer: cancer heterogeneity. Essentially, not all tumor cells are identical for biology and chemosensitivity. This is one of the reasons contributing to the treatment failure and disease progression. Surgery can successfully remove cancer from the body, while combining radiotherapy with chemotherapy can effectively give better results for treating many types of cancer [30, 31]. However, chemotherapy can also induce tumor heterogeneity, resulting in suboptimal anticancer action, ultimately contributing to treatment failure and disease progression [32, 33]. Chemoresistance is a major problem in the treatment of cancer patients, as cancer cells become resistant to chemical substances used in treatment, which consequently limits the efficiency of chemo agents [34]. It is also often associated with tumors turning into more aggressive form and/or metastatic type [35–38].

2.1.3 LC and immuno-target therapy

During the past few decades, research has provided breakthroughs that have enhanced our understanding of the mechanisms and pathways that regulate the immune system's response to cancer [39]. However, despite these advances, obstacles still exist in cancer immunotherapy [39]. These include the inability to predict treatment efficacy and patient response, the need for additional biomarkers, the development of resistance to cancer immunotherapies, the lack of clinical study designs that are optimized to determine efficacy, and high treatment costs [40–49]. Future advances in cancer immunotherapy are expected to overcome and resolve many of these challenges. A major challenge for cancer immunotherapies is the need to develop agents that are consistently effective in a majority of patients and cancer types [40]. Dramatic results have been observed in some patients treated with cancer immunotherapies, indicating that it is feasible to restore effective antitumor immune surveillance [40]. However, to date, many immunotherapy treatments have demonstrated efficacy in only a select group of cancers, and usually in a minority of patients with those cancers [41, 42, 48].

Reasons for the variability in patient response to cancer immunotherapies have been proposed, including the need to identify additional biomarkers and cancer pathways, again tumor heterogeneity, variability in cancer type and stage, treatment history, and the still largely obscure immunosuppressive biology cancer [40, 43]. Treatments that target single molecular mutations or cancer pathways have only modestly affected survival in some cancers [44]. This approach, which has been described as "reductionist," might be improved by administering drug combinations that target multiple mutations and cancer pathways [44]. In addition, a large number of the

mutations found in human tumors do not occur with meaningful regularity among different patients [50]. Therefore, immunotherapies directed at molecular mutations most likely need to be customized and patient specific in order to be more effective [50–52].

One major limitation of cancer immunotherapy is the availability of known targetable tumor-specific antigens (TSAs), also called "neoantigens," that are solely expressed by tumor cells [39, 40]. Tumor-associated antigens, which are expressed by both tumor and normal tissues, also provide an option for immunotherapy, but targeting them is likely to cause off-target toxicities and has achieved little success [39, 40].

Moreover, it is important to develop cancer immunotherapies that enhance TSA-specific T-cell reactivity [39]. Identifying biomarkers that have predictive or prognostic value for use in selecting patients who will benefit from treatment with cancer immunotherapy is a lengthy and difficult process [44]. To date, few predictive biomarkers for cancer immunotherapy treatments have been robustly validated [44]. Still, a predictive benefit has been observed for certain biomarkers with respect to response rate in patients with oncogene addicted tumors when those patients receive matched targeted immunotherapies [44]. For example, human epidermal growth factor receptor 2 amplification has been found in 20% of patients with gastric cancer; these patients have been found to exhibit a response rate of 40% to 50% when treated with the monoclonal antibody trastuzumab [44]. PD-L1 has been perhaps the most investigated biomarker with regard to potential predictive capabilities; it has been studied in numerous randomized controlled trials [44]. Evidence in different tumor types has suggested that the higher the PD-L1 expression by the tumor, the better the response rate and survival rates with PD-1/PD-L1 ICB treatment [43, 44]. Interestingly, however, it has been found that treatment benefits with PD-1/PD-L1 ICBs are not solely restricted to PD-L1-positive patients [43, 44].

However, TSAs have been extensively investigated and are thought to be a promising category of immunotherapy targets [40, 43]. The features making TSAs potentially optimal biomarkers for cancer immunotherapy include highly selective expression in tumor versus normal tissues, broad expression in a variety of human cancers of different histological origins, and remarkable "immunogenicity" allowing the induction of humoral and/or cellular immune responses in cancer patients [40, 43]. TSAs may also be optimal targets for cancer immunotherapy directed at cancer stem cells (CSCs) [40]. TSAs are expressed by CSCs and play a role in CSC differentiation and biology [40]. Most cells comprising a tumor mass are thought to result from the differentiation and cloning of a small number of CSCs that maintain and constantly "feed" the growth of the tumor [40]. With evidence that CSCs exist in many different tumors, it is imperative to identify and understand tumor antigens expressed by CSCs.

2.2 CSC role in LC

CSCs (Fig. 2.1), also known as tumor-initiating cells, have been intensively studied in the last decades, focusing on the possible source, origin, cellular markers, mechanism study, and development of therapeutic strategy targeting their pathway [53–55].

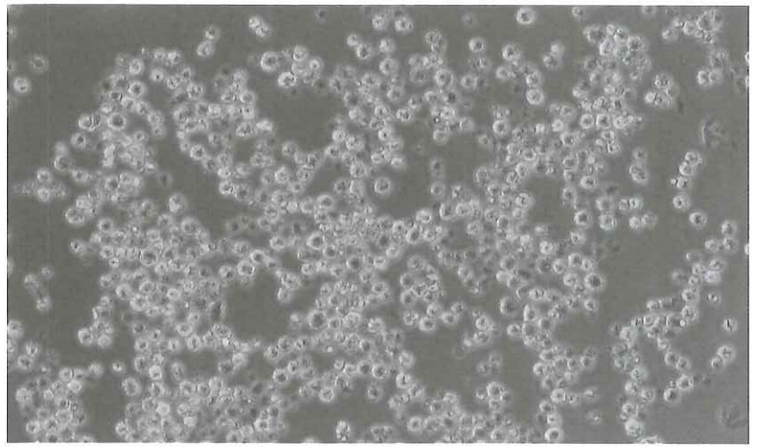

Fig. 2.1: How cancer stem cells look like before sphere formation.

In 1963, Bruce *et al.* observed [56] that only 1%–4% of lymphoma cells (not all cancer cells) can form colonies *in vitro* or initiate carcinoma in mouse spleen (Fig. 2.2). However, the first compelling evidence proving the existence of CSCs is generally acknowledged to have been provided in 1997 when scientists discovered that only the CD34+/CD38– cells from acute myeloid leukemia patients could initiate hematopoietic malignancy in NOD/SCID mice and showed that these cells had the characteristics of stemness: self-renewal, proliferation, and differentiation [55]. Thus, CSCs had the ability to differentiate into the spectrum of all cell types observed in tumors and the ability for the growth of the primary cancer tumor as well as the development of new tumors [57, 58].

CSCs are also able to induce cell cycle arrest (quiescent state) that supports their ability to become resistant to chemotherapy and radiotherapy [59–64]. Common chemotherapeutic agents target the proliferating cells to lead their apoptosis [63]. Although successful cancer therapy abolishes the bulk of proliferating tumor cells, a subset of remaining CSCs can survive and contribute to cancer relapse due to their ability to establish higher invasiveness and chemoresistance [65, 66]. Understanding the features of CSCs is important to establish the foundation for new era in the treatment of cancer. In this review, we address the detailed mechanisms by which CSCs display the resistance to chemotherapy and radiotherapy and their implication for

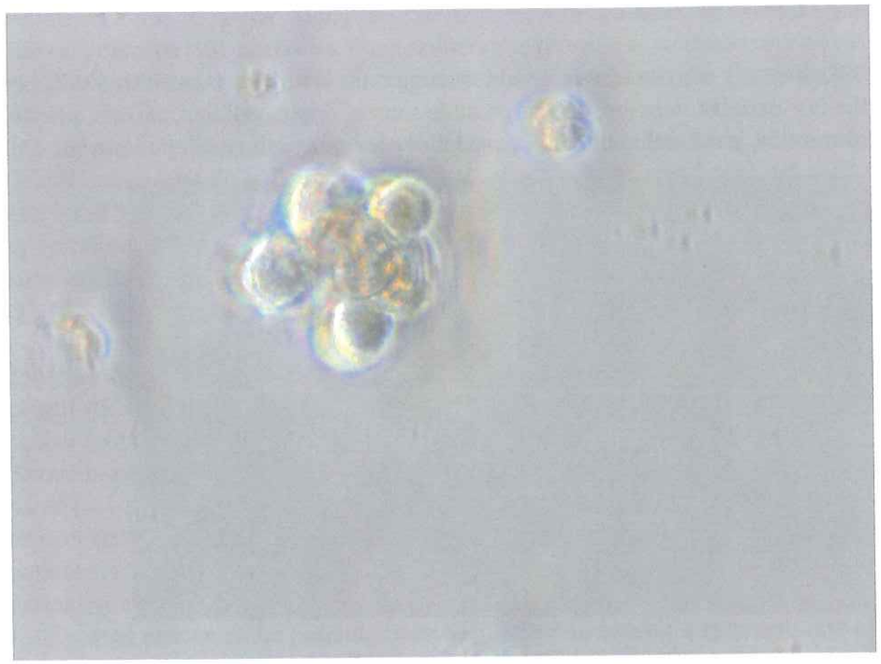

Fig. 2.2: Cancer stem cell spheroid.

clinical trials. Of note, CSCs can be identified by specific markers transferred from normal stem cells, which are commonly used for isolating CSCs from solid and hematological tumors [67]. Several cell surface markers have been verified to identify CSC enriched populations, such as CD133, CD24, CD44, EpCAM (epithelial cell adhesion molecule), THY1, ABCB5 (ATP-binding cassette B5), and CD200 [68–71]. Additionally, certain intracellular proteins also have been used as CSCs markers, such as aldehyde dehydrogenase 1 (ALDH1), which is used to characterize CSCs in many types of cancer such as leukemia, breast, colon, liver, lung, pancreas, and so forth [72, 73]. The usage of cell surface markers as CSC markers might differ from each cancer types depending on their characteristics and phenotypes (Tab. 2.1).

In recent years, there has been an increasing amount of evidence to support a CSC phenotype in human LC [74–76]. Many of these markers have also been found in other tumors and, indeed, in normal stem cells; in fact, they are now widely regarded to be stem cells in a number of malignancies, such as lung, breast, and glioblastomas, as well as in normal hematopoietic cells [77–82]. Although recent studies have contributed to a better understanding of CSC surface molecules, the picture is not yet complete. It is often observed that CSCs do not express the same markers, or that normal cells also express these surface antigens. Therefore, it is not possible yet to

Tab. 2.1: Overview of cancer stem cell markers and their functions [69].

Cell surface marker	Cancer types	Functions
CD44+	Breast, ovarian, prostate, colon, pancreatic, lung	Glycoprotein involved in migration, cell adhesion, and chemoresistance
CD24–	Breast	Down regulates the CXCR4/SDF-1 pathway
CD133	Ovarian, glioblastoma, lung, prostate, colon, renal, melanoma	Glycoprotein involved in cell growth, metastasis and chemoresistance
CXCR4	Pancreatic	Metastasis
ALDH1	Breast, head and neck, lung	CSC self-protection, differentiation, expansion, and chemoresistance

certainly isolate CSCs, but only to identify a CSC-enriched population. Consequently, identification of CSCs must be based on additional functional assays, such as the ability to form spheres in serum-free medium and to initiate tumor growth after serial transplantation in immunocompromised animal models, based on their self-renewal capacity. However, these assays also have limitations due to microenvironment. Therefore, to specifically address CSCs in further experiments, it is necessary to sort cells based on surface markers and subsequently to assess their functional abilities by *in vitro* and *in vivo* assays.

The surface markers used for the identification and isolation of CSCs are also important targets for therapy [83–91]. Immunotherapy that involves antibodies targeting CSC-specific markers is often used as an adjunct to chemotherapy, radiotherapy and surgery [92]. The most important CSC-associated markers, together with strategies for targeting them, are, for example, CD133 and CD44, even if they are not restricted to CSCs [93]. One of the mechanisms by which CSCs manage to avoid or to survive cancer treatments seems to be represented by signals generated within the tumor microenvironment, due to dysregulation of signaling pathway networks [83]. Like normal stem cells, CSCs use signaling pathways that are essential for self-renewal, proliferation, and differentiation in order to preserve stem cell properties, but the final result is carcinogenesis. Many studies have also focused on signaling pathways to deregulate CSCs attempting to find new strategy for cancer therapy; this line of research is promising mainly because many cancers present up- or down-regulation of the same signaling cascades. In this regard, CSCs can be identified by surface markers but also by the signals they send in tumor microenvironment [84]. The major involved pathways in the regulation of self-renewal and differentiation of normal and CSCs are Notch, Hedgehog, Wnt/b-catenin, nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB), phosphatidylinositol 3-kinase/ Protein kinase B (Akt), and Phosphatase and tensin homolog (PTEN); sustained by aberrant activation of these pathways, CSCs have the capacity to initiate cancer and promote recurrence after the surgical removal of tumor [85].

It has been shown that CSCs may express ABC transporters such as ABCG2, MDR1, ABCA2, etc., which have important roles in chemoresistance by active efflux of the drug from within the cell [80], and they have been successfully identified in both NSCLC and small-cell LC cell lines [74–82]. Ho *et al.* examined CSC fractions in six NSCLC cell lines and a small number of clinical samples [74]. They found that this fraction was the tumorigenic population in a xenotransplantation model requiring far fewer cells to initiate a tumor than the non-CSC fraction. Subsequent analyses of the cancer stem like cell-derived tumors also showed their differentiation into both CSC and non-CSC cells. This repopulation ability was also confirmed *in vitro*.

2.3 Role of chemotherapy and radiotherapy on CSC and tumor microenvironment

Understanding the mechanisms of chemoresistance in cancer could help to predict disease progression, develop new therapies, and personalize systemic therapy. In the last few decades, CSCs have proven to play a key role in "tumor initiation" and may also act as a key factor for chemoresistance and recurrence of the disease following chemotherapy. Resistance to anticancer therapy in patients has been attributed toward a number of factors controlling the stemness character of the CSCs that leads to therapeutic resistance.

Recent studies suggest that CSCs are enriched after chemotherapy, because a small subpopulation of cells remaining in tumor tissue that can survive and expand through most chemotherapeutic agents kill bulk of the tumors [91, 94, 95]. For instance, preleukemic DNMT3Amut hematopoietic stem cells, which can initiate clonal expansion as the first step in leukemogenesis and regenerate the entire hematopoietic hierarchy, were found to survive and expand in the bone marrow remission after chemotherapy [94]. Similarly, exposure to therapeutic doses of temozolomide, the most commonly used antiglioma chemotherapy, consistently expands the glioma stem cell (GSC) pool over time in both patient-derived and established glioma cell lines, which has been shown to be a result of phenotypic and functional interconversion between differentiated tumor cells and GSCs [96]. Therefore, by understanding the mechanisms and oncogenic drivers by which the CSCs escape radiotherapy and chemotherapy, we can develop more effective treatments that could improve the clinical outcomes of cancer patients. In order to survive during and after therapy, CSCs display many responses, including epithelial mesenchymal transition, self-renewal, tumor environment, quiescence, multidrug resistance, and dormancy. Moreover, tumor microenvironment may play a crucial role in protecting CSCs from the cytotoxic effect of chemotherapeutic drugs [97–102]. In fact, cells within the CSC microenvironment are capable of stimulating signaling pathways [82], such as Notch [103–105] and Wnt [106–108], which may facilitate CSCs to metastasize, evade anoikis, and alter divisional dynamics, achieving repopulation by symmetric division [106, 109–111].

2.4 New generation therapies based on normal stem cells to target CSC

Traditional therapies against cancer, chemotherapy and radiotherapy, have multiple limitations that lead to treatment failure and cancer recurrence. These limitations are related to systemic and local toxicity, while treatment failure and cancer relapse are due to drug resistance and survival also associated with CSC self-renewal. Therefore, in order to develop efficient treatments that can induce a long-lasting clinical response preventing tumor relapse, it is important to develop drugs that can specifically target and eliminate CSCs. Combined therapy using conventional anticancer drugs with CSC-targeting agents may offer a promising strategy for management and eradication of different types of cancers [112–114].

Besides the possibility of developing new therapies targeting CSCs, a normal population of progenitor/stem cells, namely, mesenchymal stromal/stem cells (MSCs), has been recently used as cellular vehicle for therapeutic compounds [115]. MSC can carry anticancer agents allowing a revision of the old chemotherapy-based paradigms [114]. This introduced novel therapeutic opportunities based on genetically engineered MSCs whose properties make them a unique and promising option in cancer therapy [114].

Some of the distinct properties of MSCs, such as nonimmunogenicity, stimulatory effect on the anti-inflammatory molecules, inhibitory effect on inflammatory responses, nontoxicity against normal tissues, and easy processes for clinical use, have been important prerequisites for MSCs clinical translation for cancer [114]. In 2015, Lathrop *et al.* focused the attention on malignant mesothelioma (MM), a still highly deadly lung malignancy with poor treatment options [116]. MM cells further promote a highly inflammatory microenvironment, which contributes to tumor initiation, development, severity, and propagation. This group engineered MSCs in order to overexpress tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) protein (MSC-TRAIL), which would effectively inhibit mesothelioma growth [116]. Using a mouse xenograft model of intraperitoneal human mesothelioma, native mouse (mMSCs) or human (hMSC) MSCs were administered systemically (intravenously or intraperitoneally) at various times following tumor inoculation [116]. Both mMSCs and hMSCs localized at the sites of MM tumor growth *in vivo* and decreased local inflammation. Parallel studies of *in vitro* exposure of nine primary human mesothelioma cell lines to mMSCs or hMSCs demonstrated reduced tumor cell migration [116]. However, there is an aspect that has to be better defined regarding the role of MSC in tumor tropism. This seems to be related to the type of tumor and histotype, as demonstrated by tumor development after subcutaneous coadministration of MSCs with allogeneic melanoma cells [117, 118]. This effect was attributed to the immunosuppressive effect of MSCs, which suppressed the host immune reaction to the allogeneic melanoma cells. [117]. Engineered MSC-TRAIL served as a platform for an efficient and targeted form of therapy [112, 116, 117].

In particular, TRAIL can bind to a receptor preferentially expressed on tumor cells, causing them to undergo apoptosis, and engineered MSCs have proven highly effective against cancer cell lines and animal models of cancer, including LC [112]. Recently, Janes and colleagues have developed clinical-grade engineered MSCs showing that they retain their potency even after freezing [119]. Patients with advanced metastatic LC are now being recruited for a phase I/II trial that will compare a combination of TRAIL-armed MSCs and chemotherapy with the current chemotherapy standard of care. Even if MSC-TRAIL can target CSCs, which are resistant to many conventional chemotherapies, and act synergistically with chemotherapy, the presence of TRAIL-resistant CSC clones shall be taken into account for more effective treatment [113, 120]. Nonetheless, with the discovery of small molecular inhibitors that could target CSCs and tumor signaling pathways, a higher efficacy of MSC-TRAIL-mediated tumor inhibition can be achieved [114]. This might pave the way for a more effective form of combinatory therapies, which shall lead to a better treatment outcome [113]. However, the major problem regarding LC diagnosis is that the patients received a diagnosis in an advanced-stage disease and that a large part of these patients did not survive despite treatment. Similarly, the prognosis remained poor even in locally advanced disease because of the high relapse rate and early formation of micro-metastases. CSCs were thought to be a primary obstacle to cancer therapy; for this reason, great effort has been lavished for the development of anti-CSC strategies [121]. Recently, researchers focused their attention in the delivering or administration of drugs that eliminate CSCs, which might represent a more efficient therapeutic approach for the treatment of patients with recurrent or advanced stage LC [121]. Studies have been published to find out new efficient drugs targeting CSCs, especially due to the fact that CSCs possessed drug resistance granted by their ability to actively expel therapeutic drugs *via* transport proteins, such as ATP-binding cassette. These proteins use ATP-dependent efflux pumps to eliminate drugs into the extracellular space [122]. Methods for the administration of anticancer drugs have been evaluated in order to maximize their effects, minimizing side effects in normal tissues and damage in normal stem cells. Scientists had to take into account some important aspects, such as the vascular endothelial thinness in a cancer cell with respect to normal ones, that facilitate the delivering and, in addition, the lack of an effective lymphatic drainage ensured drugs to be much more easily retained in cancer than in normal tissues. This last feature was called a retention effect used extensively in anticancer drugs modified with liposome, nanomaterials, or high–molecular weight polymers [123].

Currently, there were two methods used for discovering new efficient drugs, one was based on validation of old drugs targeting CSCs and the other one relied on a traditional method build on a high-throughput screening, which is profitable for discovering new drugs among many compounds [124]. Drugs should impact CSCs, inhibiting their self-renewal activity, inducing apoptosis, oxygen reactive species, and ALDH, moreover inactivating the ubiquitin-proteasome pathway. An example was the case of

the drug thioridazine, an antipsychotic, which selectively targets leukemia stem cells via the dopamine receptors, without being cytotoxic to normal blood stem cells [125], and its anticancer potential was also reported in breast and gastric carcinoma [126]. Studies made on disulfiram, a drug used for treating alcoholism, showed anticancer activity *in vitro* and *in vivo*, further potentiating the chemotherapeutic response. Its effectiveness has been demonstrated on paclitaxel resistant triple-negative breast cancer cells, in NSCLC cells, and glioblastoma [127, 128].

Another new branch regarding the identification of new drugs anti-CSCs is represented by oncolytic viruses, which differ from those of conventional therapies and represent a completely different class of therapeutics that can kill cancer cells in a variety of ways. Unlike radiation and chemotherapeutics, many oncolytic viruses, including vaccinia virus, adenovirus, HSV, and retrovirus, can infect both quiescent and dividing cells and replicate efficiently in those cells. Consequently, most oncolytic viruses tested against CSCs have been found to have more or less similar efficacy in killing CSCs and non-CSCs. Recent studies have shown that oncolytic viruses can efficiently kill CSCs in many types of cancer [129]. While several preclinical studies have shown that oncolytic virus as a monotherapy may be effective against some malignancies, it would be logical to combine oncolytic virus with traditional therapies to achieve greater therapeutic benefits [129]. Given the fact that oncolytic viruses and traditional therapies exert their antitumor effect through different mechanisms, one would expect to achieve additive, if not synergistic, antitumor effect from combination therapies. Indeed, several studies have shown that combination of oncolytic virus with chemotherapy or radiation therapy results in synergistic antitumor effect in animal models [130–133]. Additionally, transgenes ranging from toxic genes for direct killing of cancer cells to immune-stimulatory genes for activation of antitumor immunity could be inserted into oncolytic viruses to further increase the overall efficacy of oncolytic viruses. One major concern in the use of oncolytic virus for killing CSCs is that CSCs have many properties in common with normal stem cells. Therefore, oncolytic viruses may kill CSCs and normal stem cells to similar levels. However, several studies have shown that despite similarities between normal stem cells and CSCs, oncolytic viruses specifically kill CSCs while leaving normal stem cells unharmed [128–136].

In conclusion, CSCs in LC are important diagnostic and therapeutic targets to consider (Fig. 2.3).

They also represent a relevant cell type to study focusing on drug resistance and cancer heterogeneity accounting also for the immunological pressure due to the latest checkpoint inhibitor therapy. Other stem cell types may have to be accounted for by therapeutic purposes. This is the case of normal MSCs that are now the delivery vehicles of therapeutic genes as a new approach in the treatment of various types of cancers. In addition, the distinct properties of MSCs, such as tumor-tropism, non-immunogenicity, stimulatory effect on the anti-inflammatory molecules, inhibitory

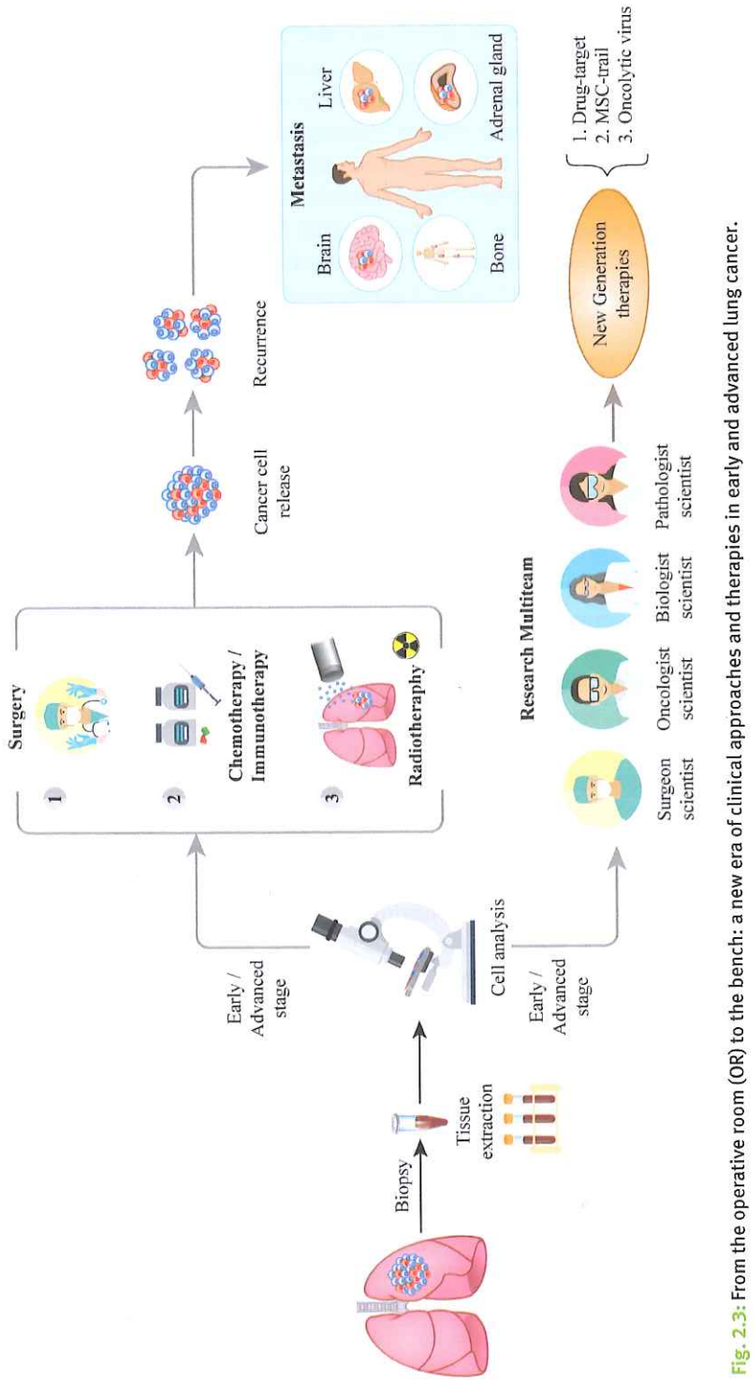

Fig. 2.3: From the operative room (OR) to the bench: a new era of clinical approaches and therapies in early and advanced lung cancer.

effect on inflammatory responses, nontoxicity against the normal tissues, and easy processes for clinical use, have set the basis of their attention.

2.5 Funding

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