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Cancer Stem Cells and the Ontogeny of Lung Cancer

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Abstract

Lung cancer is the leading cause of cancer death in the world today and is poised to claim approximately 1 billion lives during the 21st century. A major challenge in treating this and other cancers is the intrinsic resistance to conventional therapies demonstrated by the stem/progenitor cell that is responsible for the sustained growth, survival, and invasion of the tumor. Identifying these stem cells in lung cancer and defining the biologic processes necessary for their existence is paramount in developing new clinical approaches with the goal of preventing disease recurrence. This review summarizes our understanding of the cellular and molecular mechanisms operating within the putative cancer-initiating cell at the core of lung neoplasia.

INTRODUCTION

At more than 1 million deaths per year, lung cancer represents the leading cause of cancer mortality worldwide.¹ Thanks largely to Doll and Hill's seminal paper,² and the publication of the Surgeon General's report in 1964, the long-held suspicion of a connection between tobacco smoking and lung cancer is today readily acknowledged. It is predicted that, of the approximately 10 million deaths caused by smoking annually by the year 2030, one quarter will be attributable to lung cancer.³

Despite the marked improvement in the survival of patients with other common malignancies (eg, breast, prostate) arising from advances in diagnostics, surgery, and chemotherapy, overall 5-year survival rates in all lung cancer has changed little in recent decades, from figures of less than 9% in developing countries to 15% in the United States.^{1,4} The picture is even bleaker for the 15% to 20% of lung cancer patients who present with small-cell lung cancer (SCLC),⁵ which is a more aggressive malignancy than non-small-cell lung cancer (NSCLC), characterized by early dissemination. Despite initial sensitivity to chemotherapy and radiotherapy, overall 5-year survival in SCLC patients is less than 5%.⁶ As these 5-year survival statistics suggest, recurrence is common among lung cancer patients, and often refractory to the first-line therapies initially employed to treat the disease.

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One approach to improving outcome in lung cancer is to search for a unique, phenotypically defined cancer stem-cell population. Such cells, which are one potential source of tumor recurrence,⁷⁻⁹ might exhibit distinct therapeutic vulnerabilities that can be exploited as a way of controlling or eradicating minimal residual disease in lung cancer.

CANCER STEM CELLS

Only cells with long life spans are likely to accrue the requisite number and variety of genetic alterations necessary to acquire full tumorigenic capacity.¹⁰ Normal stem cells are defined by a number of characteristics that highlight the candidacy of this population as an ideal cancer-initiating cell, including longevity via their critical property of self-renewal, a capacity for extensive proliferation via transit-amplifying daughter cells and multipotency.¹¹

Far from being a new concept, the cancer stem cell (CSC) hypothesis has developed from the earlier observation that, not unlike normal adult tissues, cancer consists of heterogeneous cell phenotypes of which only a small subset can fully regenerate the tumor.⁷⁻⁹ Initially described in myeloid leukemia,¹² this idea functionally divides cancers into two distinct populations; a relatively well-differentiated subset with limited proliferative capacity, which forms the bulk of the tumor and phenotypically characterizes the disease, and a second smaller, less differentiated subset that contains clonogenic CSCs. Thus, the chief translational repercussion of the CSC hypothesis is the need to specifically target therapies at the self-renewal capacity of the stem-cell compartment, effectively interrupting the source of recurrence in tumors sensitive to conventional therapeutic approaches.

Possession of multiple-drug resistance is an additional property of normal stem cells that contributes to their longevity by permitting them to survive toxic insults, including many of the drugs currently used to treat cancer. This is often mediated by the overexpression of adenosine triphosphate-binding cassette (ABC) transporters that efflux drugs out of the cell, and conservation in CSCs is the likely cause of chemoresistance.^{7,8} In the absence of specific surface antigens, this property has been used as a surrogate marker to identify stem/progenitor cells in normal tissues,¹³⁻¹⁶ including the lung.^{17,18} Similarly, populations with characteristics of stem/progenitors cells have also been differentially identified on the basis of their survival in lung-injury models.¹⁹⁻²² These initial efforts to identify an airway epithelial stem cells are summarized elsewhere.^{23,24}

STEM CELLS IN HEALTHY LUNG

Kim et al²⁵ have expanded on these earlier studies to describe a rare population of bronchioalveolar stem cells (BASCs) in adult mice. These were initially identified by their expression of both the alveolar epithelial type II (AT2) cell marker, surfactant protein C (SP-C), and the bronchiolar epithelial Clara cell marker, Clara cell secretory protein (CCSP). This approach was prompted in part by the observation that almost all cells present in the epithelium of the smaller, distal airways during early embryonic lung development (E13-E15), express markers of AT2, Clara, and neuroendocrine (NE) cells.²⁶ These BASCs were found to be restricted to the bronchioalveolar duct junction (BADJ). This is the same marginal region between terminal bronchial and alveolar epithelium, where another putative stem-cell niche was previously identified, containing "variant" Clara cells with an inherent resistance to naphthalene-induced airway injury and the capacity to contribute to subsequent distal airway epithelial repair.²² BASCs demonstrated a similar resistance to naphthalene-induced toxicity and increased in number during the period of bronchiolar epithelium repair.²⁵ In addition to the intracellular proteins SP-C and CCSP, BASCs were also found to coexpress Sca-1 and CD34 antigens, both of which are established surface markers of stem-cell populations in other mouse tissues.^{27,28} By excluding endothelial (CD31+) and hematopoietic (CD45+) cells, viable BASCs were able to be isolated by flow cytometry and

carried over multiple passages in vitro.²⁵ Under distinct culture conditions, these purified BASCs could be induced to either expand while maintaining their undifferentiated state (SP-C–positive/CCSP-positive), or give rise to progeny with Clara cell (SPC–negative/CCSP-positive), AT2 (SP-C–positive/CCSP-negative), or AT1 (SP-C–positive/aquaporin5-positive) phenotypes.²⁵ This connects BASCs to the core stem-cell properties of self-renewal and multipotent differentiation, but the ultimate demonstration of stem-cell pedigree still remains to be performed in vivo.

More recently, another putative lung stem-cell population has been identified in cytokeratin-positive epithelial colonies arising from in vitro culture of mouse primary neonatal whole lung cells in serum-free conditions.²⁹ These colonies are CCSP-positive and Sca-1–positive, but differed from BASCs by virtue of their negative expression of SP-C and CD34.²⁹ In addition to Sca-1, these epithelial colonies also express Oct-4 and SSEA-1, two markers of self-renewal and pluripotency in embryonic stem cells.³⁰ BrdU pulse-chase experiments performed in neonatal mice localized a small population of slow-cycling, Oct-4–positive cells in the epithelial layer of the BADJ.²⁹ Considering the similarities between these two putative lung stem-cell populations, it remains a possibility that BASCs are descended from these Oct-4–positive neonatal lung cells.²³

The multilineage differentiation capacity of individual Oct-4–positive colonies was demonstrated after transfer of individual, primary colonies to culture dishes coated with collagen I. This seemed to result in a sequential differentiation process, characterized by an initial loss of “stemness” (ie, Oct-4 protein) and subsequent maturation of an AT2 cell phenotype (SP-C–positive) that in turn generated an AT1 (aquaporin5-positive) population.²⁹ This result partly emulates that of an early study in which AT2 cells were proffered as a putative stem/progenitor population, in response to their expansion and apparent role in repair after bleomycin-induced ablation of AT1 airway epithelial cells.¹⁹ Since the presence of Oct-4–positive cells was not assessed over multiple passages, in either the primary or differentiating culture conditions employed in this article,²⁹ it precludes an assessment of the self-renewal capacity of this putative stem-cell population.²⁹ Interestingly, the authors also reported that the Oct-4 –positive epithelial colonies are permissive for infection by the variant coronavirus responsible for severe acute respiratory distress (commonly called SARS), and suggest that the severity of this disease may result from the loss of this putative stem-cell compartment, which prevents replacement of specialized lung epithelial cells also damaged by the virus.²⁹

BASCs AND TUMOR SUPPRESSORS

In three separate studies published in 2007, homeostatic regulation of the BASC compartment was linked to expression of several well-characterized tumor suppressor genes.^{31–33}

A widely expressed member of the mitogen-activated protein kinase (MAPK) family, p38α (Mapk14) is believed to regulate the differentiation and proliferation of cells in a variety of different mammalian tissues.³⁴ Now there is compelling evidence to indicate that p38α plays a key role in the division and differentiation of lung epithelium stem/progenitor cells.³¹ Comparison of adult mice heterozygous (*Mapk14* positive/negative) or homozygous (*Mapk14* negative/negative) for a *p38α* conditional knockout allele, revealed that lungs from the latter possessed hyperproliferative alveolar epithelium, which was associated with greatly elevated numbers of SP-C–positive cells exhibiting a lack of differentiation markers.³¹ These *p38ba* knockout mice eventually developed respiratory problems and died prematurely. Many of the SP-C–positive cells were identified as a cell-cycle–arrested, immature AT2-like population. However, costaining with CCSP and Sca-1 also revealed an

expanded BASC (SP-C–positive/CCSP-positive/Sca-1–positive) subset within this population,³¹ whose niche was no longer restricted to the discrete BADJ region described previously.²³ A higher proportion of the BASCs isolated ex vivo from *Mapk14*-negative/-negative lungs exhibited a more active cell-cycle profile than from *Mapk14*-positive/-negative mice, and divided more rapidly in vitro.³¹ Intensifying this contrast, p38 α -null BASCs cultured under differentiation-permissive conditions retained their SP-C–positive/CCSP-positive phenotype, instead of differentiating into both SP-C–positive or CCSP-positive progeny. *Mapk14*-positive/-negative BASCs could be persuaded to behave like their *Mapk14*-negative/-negative counterparts in vitro by treatment with a p38 α inhibitor, associated with increased epidermal growth factor receptor and decreased C/EBP α protein levels, respectively.³¹

Unsurprisingly, levels of C/EBP α and the downstream target, HNF3 β were low in *p38 β* -null lungs. Both transcription factors are necessary for the differentiation of stem cells into more mature lung progeny,^{35,36} perhaps explaining the poor differentiation capacity of BASCs in these lungs. Similarly, p38 α has an established role in inhibiting CyclinD1 activity³⁷ and seems responsible for regulating epidermal growth factor receptor levels in the lung,³¹ which might help to explain the enhanced proliferation of BASCs in *Mapk14*-negative/-negative mice.

Cyclin-dependent kinases (CDKs) play a key role in regulating the cell cycle by integrating various signaling pathways of G₁ cell-cycle control. They themselves are negatively regulated via binding by members of the INK4 family, including *p18* (*Ink4c*). Indeed, p18 is recognized as limiting the self-renewal capacity of hematopoietic stem cells,³⁸ a role that seems to extend to BASCs because an expanded SP-C–positive/CCSP-positive population has been observed in the BADJ of lungs from young *p18*-negative/-negative mice.³² Nevertheless, the surrounding lung architecture did not seem to be affected.³² A similar expansion of BASCs was described in the lungs of mice with a bronchioalveolar epithelium-specific null mutation of phosphatase and tensin homolog 10 (*Pten*),³³ a key regulator of cell division whose absence is associated with stem-/progenitor- cell expansion in a number of tissues, and a tumor suppressor that is mutated at high frequency in a large number of cancers.³⁹ Interestingly, BASC hyperplasia was associated with dramatically increased expression of Sonic Hedgehog (Shh) in the lung, a molecule that is required for branching morphogenesis during embryonic development⁴⁰ and promotes stem-cell self-renewal in a number of tissues.⁴¹ The Hedgehog pathway will be discussed in more detail later herein.

BASCs AS TARGETS OF TUMORIGENESIS

The double-positive, SP-C–positive/CCSP-positive phenotype used to identify BASCs was first described in the precursor lesions of a mouse model of adenocarcinoma, using an inducible allele of oncogenic *K-ras* that promotes cell proliferation.⁴² Compared with controls, numbers of BASCs were expanded in the lungs of mice in which *K-Ras* had been activated (*K-Ras*–positive), and bore a direct correlation to the accompanying tumor burden.²⁵ Hence, further expansion of the BASC pool by airway injury, before *K-Ras* activation, increased tumor size and number in this model.²⁵ Similarly, BASCs from *p38 α* -null mice were also sensitized to *K-Ras*-induced lung tumors, permitting earlier induction and faster progression to adenocarcinoma.³¹ This finding is accompanied by the observation that human lung tumors express lower levels of p38 α than are found in normal lung tissue.³¹ Because these adenocarcinomas contained populations consistent with the presence of both AT2 and AT1 cells,⁴² it seems that BASCs expressing activating *K-Ras*–positive mutation retain a capacity for differentiation similar to their normal counterparts, a conclusion supported by in vitro studies.²⁵ In a related model, the BASC expansion generated in *p18*-negative/-negative mice can collaborate with a *Men1*-positive/-negative model to create

more aggressive and heterogeneous tumors, respectively, than each model alone.³² The *Men1* gene encodes menin, a tumor suppressor that regulates cell-cycle progression.⁴³ Similarly, the initially mild bronchioalveolar hyperplasia associated with the early expansion of BASCs in *Pten*-null mice progressed to spontaneous lung adenocarcinoma. Moreover, *Pten*-null mice were more susceptible to chemically-induced carcinogenesis.³³ These findings are particularly relevant because recent studies demonstrated decreased PTEN expression in some 70% of human NSCLC, mostly in response to epigenetic mechanisms like promoter methylation.⁴⁴

Yet a further illustration of BASCs as lung cancer precursors was reported for mice in which a modified *p27* (*Cdkn1b*) gene (p27 CK-negative) had been knocked in. In these mice, the CDK-dependent tumor suppressor activity of p27 was eliminated, revealing a CDK-independent, oncogenic function that resulted in hyperplasia and tumorigenesis in multiple organs.⁴⁵ In the lungs, this was initially manifest as an expansion of BASCs at the BADJ and later throughout the bronchioalveolar epithelium. With increasing age of the mice, this progressed from hyperplasia to dysplasia to development of adenomas and, finally, overt tumors with features of adenocarcinomas.⁴⁵

In addition to bolstering the hypothesis that lung stem/progenitor cells are the targets of tumor initiation and sustain the tumor throughout its existence, these studies support the assertion that expansion of stem/progenitor cells alone is not sufficient for tumorigenesis, but requires at least one further genetic or epigenetic event. Furthermore, it would seem that this genetic insult can assume a variety of different forms and it will be interesting to learn whether other mouse models of lung cancer feature or can cooperate with early expansions of BASCs. It would be particularly beneficial to know whether BASCs can contribute to the development of tumor subtypes other than adenocarcinoma, such as in the conditional *Rb1/Trp53* knockout model of SCLC.⁴⁶ These tumors contain markers of NE differentiation that have not been reported as being generated by normal lung BASCs.²⁵

HUMAN LUNG CSCs

The presence of a clonogenic population of cells in human lung cancer was first described more than 25 years ago.⁴⁷ Clinical specimens from both adenocarcinoma and SCLC patients contained small populations of cells (< 1.5%) that were able to form colonies in a soft agar cloning assay. When individually-selected soft agar colonies were injected intracranially into athymic nude mice, they were able to generate tumors with features characteristic of the original patient specimen,⁴⁷ thus supporting the existence of a CSC population in these malignancies.

Small numbers of side-population (SP) cells, identified by the efflux of Hoechst 33342 by ABC transporters, have been shown to possess stem-cell characteristics.¹⁵ SP cells have recently been identified in both NSCLC clinical samples and cell lines, the latter of which expressed elevated transcripts of several ABC transporters.⁴⁸ This was associated with an enhanced resistance to a number of commonly used chemotherapeutic agents, compared with non-SP cells.

Consistent with the belief that CSCs are an immortalized and largely quiescent population,⁹ SP cells also contain elevated mRNA levels of the cellular senescence inhibitor, telomerase, and decreased mRNA levels of the proliferation marker, *Mcm7*, respectively.⁴⁸ In vitro, purified SP cells demonstrated a higher potential for invasiveness and were able to generate both SP and non-SP subsets, such that the resulting culture resembled the original, presort cell line. In vivo, SP cells were enriched for tumor-initiating capacity relative to the non-SP population, requiring substantially smaller inoculums to generate tumor xenografts in nonobese diabetic/severe combined immunodeficiency mice.⁴⁸

Because of the implications for cancer treatment, considerable effort is being expended in the search for robust markers of CSCs, particularly those expressed extracellularly and independent of functional stem-cell assays for their identification and purification. This is paramount to accurately delineate the mechanisms responsible for the transformation of normal lung cells into CSCs.⁴¹ Attempts to identify a phenotypic marker for the Hoechst dye-excluding compartment described in human NSCLC were unsuccessful, given that additional staining for CD24, CD34, CD44 and nestin failed to differentiate between SP and non-SP populations.⁴⁸ In human SCLC cell lines, a small population of urokinase-type plasminogen activator (uPAR)-positive (CD87) cells was identified, in which a subset demonstrated enhanced clonogenic activity in vitro.⁴⁹ The presence of both uPAR-positive and uPAR-negative cells in the resultant colonies suggested a multilineage potential for this putative stem cell, but this was not characterized in any greater detail. Purified uPAR-negative cells from SCLC lines demonstrated little or no clonogenic activity.⁴⁹ uPAR is linked to regulation of cell adhesion and migration, and elevated levels have been associated with a poor clinical outcome in a variety of tumors.⁵⁰

Putative CSC markers that have been described for other malignancies, including acute myeloid leukemia (CD34-positive/CD38-negative),¹² breast (CD44-positive/CD24-negative/-low/Lin-negative),⁵¹ prostate (CD44-positive/ α 2 β 1-high/CD133-positive)⁵² and brain (CD133-positive/nestin-positive),⁵³ have reflected those expressed by their normal tissue counterparts. Unfortunately, the identification of a human BASC counterpart, which might represent an important first step, may be difficult by virtue of the fact that of the two surface markers used to define mouse BASCs,²⁵ Sca-1 has no known human homolog and CD34 staining does not correspond to the putative SP stem cell reported in NSCLC.⁴⁸ However, a very recent report describes a rare population of CD133-positive cells in normal mouse lungs that, like BASCs, undergoes significant expansion during the regeneration of naphthalene-injured airways.⁵⁴ Freshly dissociated human SCLC and NSCLC tumors contained a similar CD133-positive subset. These cells were able to generate long-term lung tumor spheres in vitro that could both differentiate and preferentially form tumors in vivo.⁵⁴ Chemoresistance in the tumor spheres was associated with expression of the ABC transporter responsible for the SP phenotype, as well as the embryonic stem-cell markers OCT4 and NANOG.⁵⁴ Despite the commonality of CD133 expression in different subtypes of lung cancer, it remains to be confirmed whether a single, normal lung stem/progenitor phenotype is sufficient to define the CSC in each case, or if multiple origins are indicated.

EMBRYONIC PATHWAYS AND LUNG CSCs

The idea that embryonic development programs play key roles in lung tumorigenesis is supported by a recent study in which the molecular signatures of different human lung cancer subtypes were compared against the changing expression profiles of murine orthologs at different stages of mouse lung development.⁵⁵ Unsurprisingly, normal human lung samples were most similar to late mouse lung development stages. In placing samples from three human lung cancer subtypes along this timeline, the most aggressive malignancy, SCLC, appeared the earliest, followed by squamous carcinoma and then adenocarcinoma, reflecting their increasing 5-year survival rates. Put another way, the earlier the stage of mouse lung development that a patient's lung cancer resembled, the shorter their survival time.⁵⁵

More than 150 years ago, Rudolf Virchow published his theory, "*Omnis cellula e cellula*" ("Every cell originates from another existing cell like it"), in which he proposed that cancer arises from embryonic-like cells.⁵⁶ This view has undergone a revival as the CSC theory. There is growing evidence that abnormal activation of embryonic patterning pathways, such as Notch and Hedgehog, are responsible for the expansion and malignant transformation of

normal stem cells, representing an early event in tumorigenesis.^{7–9} These pathways are important regulators of cell fate decisions in the developing lung and their contribution to malignancies arising in this organ are discussed.

Notch Pathway

One potential outcome of the cell-to-cell interactions mediated by the Notch family of receptors relates to binary cell fate decisions, with Notch activation favoring one fate over another. This may represent a choice by daughter cells between one of two new fates, or alternatively, adopting a new fate versus retaining its original status.⁵⁷ Thus, a critical Notch function in both development and homeostasis is the maintenance of stem-cell viability via asymmetric cell division, as demonstrated for stem cells in the bone marrow, brain, intestinal crypts and breast epithelium.⁴¹ In a simplified overview of Notch signaling, receptors on one cell are activated by ligands on an adjoining cell, leading ultimately to the activation of transcriptional targets, principally a set of inhibitory basic helix-loop-helix transcription factors that includes *Hes1*. In turn, these transcriptional repressor proteins obstruct positively-acting basic helix-loop-helix members.⁵⁷

Knockout mouse studies demonstrate a requirement for Notch signaling in lung development.⁵⁸ Elevated Notch ligand, receptor and *HES1* levels have been demonstrated in NSCLC lines,^{59,60} and Notch signaling also seems to be among key downstream effectors of oncogenic RAS,⁶¹ which is commonly present in this malignancy. The relevance of Notch signaling to NSCLC pathogenesis is highlighted by data demonstrating increased apoptosis and serum dependence, and reduced in vitro and in vivo NSCLC tumor growth, in response to blockade of pathway activation using a γ -secretase inhibitor.⁶⁰ Paradoxically, overexpression of activated Notch-1 inhibited the in vitro clonogenicity and in vivo tumorigenicity of the A549 adenocarcinoma line,⁶² underscoring the complexity of the Notch pathway and the varying roles it may play in different lung tumor subtypes.

HES1 ordinarily blocks the transcription of human achaete-scute homolog-1, which is highly expressed in SCLC, a tumor with characteristic NE features.⁶³ Lungs from *Hes1* knockout mice have markedly increased NE differentiation with a commensurate decrease in Clara cells.⁶⁴ Notch receptor expression is rarely seen in SCLC, but overexpression of activated Notch receptors inhibited SCLC growth.⁶⁵ Activation of Notch-1 signaling produced antitumor effects in another neuroendocrine tumor, pulmonary carcinoids, which was associated with reduced levels of human achaete-scute homolog-1 protein.⁶⁶ Hence, the hypothesis here seems to be that defects in the Notch pathway facilitate the maintenance of a primitive NE phenotype.

Hedgehog Pathway

The Hedgehog pathway has been shown to be essential for normal mammalian development.⁶⁷ Described simply, signaling via this pathway in mammals is initiated by the binding of the secreted morphogen (Shh, Ihh, and Dhh) to its receptor, Patched (Ptch), relieving the repression of Smoothened (Smo) and ultimately manifesting itself in the regulation of the Gli transcription factors (Gli1, Gli2, and Gli3) and downstream target genes. Hedgehog-interacting protein was shown to attenuate Hedgehog signaling via direct binding to Hedgehog ligand, thereby enabling a differential response in an otherwise competent population of cells.⁶⁷ It is apparent that Hedgehog signaling is important in early lung formation.⁶⁸ Epithelial-mesenchymal tissue interactions control the branching of the developing mouse lung buds, and it is here that *Shh* is expressed at the highest levels, although low levels, which decline toward birth, are observed throughout the epithelium.⁶⁹ *Ptch* and the three *Glis* are expressed in the adjacent lung mesenchyme and levels reflect those of *Shh* expression. *Shh*-null mice exhibit serious foregut defects, including incomplete

separation of the respiratory and digestive systems and the appearance of two small, hypoblastic lung buds containing sac-like structures but no airway branching.^{40,70} Conversely, the overexpression of *Shh* under control of an SP-C promoter results in an absence of functional alveoli, and a hyperproliferation of both epithelial and mesenchymal lung cells.⁶⁹

In the adult, constitutive Hedgehog signaling seems to be restricted to small numbers of cells persisting in the basal layer of the bronchial epithelium at low levels.⁷¹ Airway regeneration, induced after naphthalene injury, resulted in expansion of an intraepithelial cell population with active Hedgehog signaling. This pathway reactivation preceded the expansion of a normally rare airway NE population,⁷¹ which is considered a potential progenitor of epithelial regeneration.²⁰ Using confocal immunofluorescence, similar clusters of epithelial cells expressing Ptch and the NE marker, C_{GRP}, were observed in embryonic mouse airways, immediately adjacent to cells expressing Shh.⁷¹ Unlike NSCLC lines, the growth of SCLC, a tumor with primitive features of pulmonary neuroendocrine differentiation and retention of components of the Hedgehog signaling pathway, was inhibited by the steroidal alkaloid Hedgehog antagonist, cyclopamine.^{71,72} Together, these data suggested that normally, airway epithelial progenitor cells can assume an NE fate in response to Shh signals provided by adjacent epithelial cells, but that SCLC may be the result when this signal is unregulated.

CONCLUDING REMARKS AND THE FUTURE

The tenet currently guiding research in our laboratory is predicated on the existence of a small chemoresistant population of clonogenic cells within SCLC, which are dependent on aberrant activation of Hedge-hog signaling for their preservation. As such, CSCs at the source of this malignancy should be susceptible to elimination or control through the use of Hedgehog pathway antagonists. In the absence of a well-accepted stem-cell marker in SCLC, we initially investigated this idea in multiple myeloma (MM), a malignancy with a well-defined stem-cell compartment that is phenotypically distinct from the differentiated plasma cells that pathologically define the disease.⁷³ In this model, we showed that Hedgehog pathway activity was markedly concentrated within the MM-CSC compartment, and that it was responsive to pathway modulation.⁷⁴ As such, Hedgehog pathway blockade markedly inhibited clonal expansion accompanied by terminal differentiation of purified MM-CSC, while having little or no effect on malignant plasma cell growth. Treatment with Hedgehog ligand, by contrast, promoted expansion of MM stem cells without differentiation.⁷⁴ Similar results have recently been described in glioblastoma.^{75–77}

In preliminary studies, we have used the Aldefluor reagent system to identify a small population in several SCLC cell lines with high-level aldehyde dehydrogenase (ALDH) activity, a functional property of stem cells.¹⁶ The clonogenicity of purified ALDH-high cells in an in vitro assay is superior to that of unsorted SCLC samples, but is inhibited by Hedgehog antagonists (unpublished data). ALDH1 expression has been identified as a marker of self-renewing mammary stem cells with broad differentiation potential, in both normal and malignant tissues.⁷⁸ Moreover, a retrospective analysis of breast carcinoma tissue microarrays revealed that positivity for ALDH correlated with a poor prognosis.⁷⁸ Similarly, immunohistochemical analysis has shown elevated expression levels of two ALDH isozymes in several NSCLC subtypes compared with control lung tissue.⁷⁹ In fact, even in non-neoplastic pneumocytes, cigarette smoking alone seems sufficient to elevate ALDH expression.⁷⁹

We are now pursuing a number of approaches to prospectively identify a surface marker for our putative lung CSCs, which will assist us in demonstrating the significant overlap between clonogenic, Hedgehog pathway active and chemoresistant populations within

SCLC. Interestingly, after exposure to cigarette smoke in vitro, human bronchial epithelial cells acquire the ability to form carcinomas in athymic nude mice, which is directly associated with Hedgehog pathway activation.⁸⁰

Therapeutic targeting of embryonic signaling pathways presents a new paradigm for pharmaceutical development. This approach needs to take into consideration the minor contribution of the target cell to the total tumor burden and develop appropriate assays by which true drug efficacy can be measured.

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