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Stem cell and lung cancer development: blaming the Wnt, Hh and Notch signalling pathway

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Abstract Primary lung cancer may arise from the central (bronchial) or peripheral (bronchiolo-alveolar) compartments. However the origins of the different histological types of primary lung cancer are not well understood. Stem cells are believed to be crucial players in tumour development and there is much interest in identifying those compartments that harbour stem cells involved in lung cancer. Although the role of stem cells in carcinogenesis is not well characterised, emerging evidence is providing new insights into this process. Numerous studies have indicated that lung cancer is not a result of a sudden transforming event but a multistep process in which a sequence of molecular changes result in genetic and morphological aberrations. The exact sequence of molecular events involved in lung carcinogenesis is not yet well understood, therefore deeper knowledge of the aberrant stem cell fate signalling pathway could be crucial in the development of new drugs against the advanced setting.

Keywords Stem cells · Embryonic signalling pathway · Lung carcinogenesis

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Introduction

Lung cancer is the leading cause of cancer-related death in the world [1]. During the last two decades, several chemotherapeutic and molecular target driven agents against angiogenesis and epidermal growth factor (EGF) pathway have been tested in non-small-cell lung cancer (NSCLC) with success [2]. More recently it has been found that approximately 3–4% of NSCLC patients are driven by a fusion protein of the anaplastic lymphoma kinase (ALK). By targeting this intracellular aberration with selective inhibitors we achieve an impressive objective response rate and prolonged progression-free survival figures, but resistance seems to reappear sooner or later [3]. Despite greater knowledge of the molecular biology underlying lung tumorigenesis, we are far from cure and median overall survival for metastatic diagnosed patients barely reaches 12 months.

The concept of cancer stem cell is relatively new when applied to solid tumour development. The elucidation of embryonic signalling as a vital process for self-renewal was first shown in haematopoietic stem cells and later in leukaemias [4]. As much as we are aware of the different routes that govern cancer stem cells, we are able not only

to identify but also to target these stem cells. Whether we could effectively target these cells and this could lead to tumour stasis needs to be checked in randomised trials.

Here we review the current knowledge on the role that stem cells may have in lung tumour development and the pathways involved that could be selected for targeting.

Stem cells in the respiratory system: Do they exist and if so where are they?

Lungs are composed of two primary tissue layers, namely epithelium and mesenchyme. Interactions between these two layers have been demonstrated to be essential for the sequential events of organogenesis: determination, growth, morphogenesis and cyto-differentiation [5]. The concept that these associated cell components act on each other to generate new and diverse cell types during organogenesis is termed *embryonic induction* [6].

The respiratory organ consists of several segments: the larynx, trachea, bronchi, bronchioli, respiratory bronchioli and alveoli. Each segment possesses its own specific anatomical structures as well as those common to all of them.

Advances in stem cell biology support that, as in other organs, proliferative stem cells contribute to maintenance of the epithelium in the respiratory tract. The existence of multiple progenitor cell types within the bronchial epithelium and the hierarchy with which they participate in epithelial maintenance are of fundamental importance to the understanding of mechanisms that may lead to epithelial remodelling [7, 8].

Progenitor cells responsible for maintenance of the airway epithelium have been identified in both the steady-state lung and following injury induced by mechanical or chemical disruption. Studies have identified multiple epithelial cell types with proliferative capacity and indicate that the pseudo-stratified epithelium of tracheobronchial airways is maintained by multiple progenitor cell populations that are distinct from those that sustain the simple epithelium of bronchioles [9, 10].

The multipotent pulmonary epithelial stem cells are able to differentiate into ciliated, secretor, intermediate and basal cells, and generate the submucosal glands. This variation in structure of the respiratory epithelium has raised several questions regarding the probable site for a stem cell niche. Although their specific niche and stem cell markers have not yet been established precisely [11–13], it has been proposed that these stem cells reside within the surface airway epithelium [14].

Stem cell research in the lung has progressed rather slowly due to the anatomical and functional complexities associated with numerous distinct cell types. This organ must be divided into various anatomical regions when considering multipotent progenitor or stem cells. Evidence suggests that multipotent progenitors of the conducting airway epithelium and gas-exchange alveolar regions are derived from different populations of stem cells that are anatomically separated in the lung. Stem cell niches in the conducting airways [15–17] must also be uniquely divided between the proximal and distal regions.

In the trachea, several niches [17] of stem cell expansion areas that are marked by distinct zonal boundaries have been isolated. In the proximal glandular containing trachea [18], label-retaining cells (LRCs) expanding zones were confined to the ducts of submucosal glands, while in more distal trachea and bronchi, which do not contain glands, these LCR expanding stem cells were located in systematically arrayed foci along the surface airway epithelium.

These foci in the distal trachea also appeared to be localised at cartilage–intercartilage junctions. In the distal non-cartilaginous bronchioles, LRC were also associated with neuroendocrine epithelial bodies (NEBs). These NEBs may serve as a niche in the distal trachea and bronchiolar airway for maintaining a stem cell source [19, 20].

Despite the identification of progenitor cell populations that contribute to maintenance and renewal of the pulmonary epithelium, the existence and identity of airway stem cells remains elusive. Stem cells with the potential to contribute to the reestablishment of the normal bronchiolar epithelium have not been definitively demonstrated. Two candidate stem cells were identified on the basis of proliferative potential after chemical ablation: a pollutant-resistant subpopulation of Clara cells that retain their expression of Clara cell secretory protein (CCSP), variant CGRP-expressing (CE) cells or vCE cells, and calcitonin gene-related peptide (CGRP)-expressing pulmonary neuroendocrine cells (PNECs) [21].

Characteristics that are invariably associated with the stem cell pool include a relatively undifferentiated phenotype, infrequent proliferation in the steady state, self-renewal capacity and ability to produce daughter cells capable of functioning as transit amplifying (TA) cells. Progenitor or TA cells have the capacity for only a finite number of cell divisions, have more limited differentiation capacity and generally fulfil certain differentiated functions. Several epithelial cell types have been identified in conducting airways that have properties of TA cells: these include Clara cells (the major TA source cells), basal cells and PNECs. Clara cells and PNECs are co-localised predominantly within the airway bifurcation zone of bronchioles.

Since stem cells may occupy microenvironmental niches that protect from differentiation and allow self-renewal for the life of the organism, they proliferate only during extreme injury and undergo asymmetric division into daughter stem cells and TA cells that further proliferate and differentiate.

The epithelial stem cell niche provides the protective environment for the stem cell to promote its overall survival and protects its genetic code. In rapidly renewing epithelia, the stem cell resides in a physically defined region, whereas in epithelia that does not frequently turn over (i.e., persistent epithelia), the niche is less well defined.

Regardless of the rate of turnover, the epithelial stem cell remains anchored within its niche and rarely divides. When it does divide, the stem cell does so in a symmetric fashion, renewing itself and giving rise to a daughter cell. The daughter cells are often referred to as the TA population. This TA population may proliferate more frequently than the anchored stem cell; they can travel down to the terminal differentiation and they can also dedifferentiate to replace the stem cell niche if damaged. Although the stem cell remains anchored within the niche and rarely divides, the TA population receives signals to rapidly proliferate and differentiate along one of several terminal differentiation fates.

Dissecting the regulatory pathways of stem cells in lungs

The stem cell niche encompasses a mesenchymal component: the epithelium directly contacts the underlying mesenchymal layer, and this mesenchyme is comprised of extracellular matrix, neurons, blood vessels, intraepithelial lymphocytes and fibroblast. Mesenchymal cells secrete factors that influence the overlying epithelium. Although it is clear that the cells in the mesenchyme are responsible for communicating with the overlying epithelium, the exact cells that secrete all of the important signalling factors have not been identified. Although many of the factors originate in the mesenchymal compartment of the lung, the epithelium also participates in this balanced crosstalk.

Although every single epithelial stem cell niche possesses unique features to facilitate its specialised functionality, they share many common aspects of regulation. Several signalling pathways like Wnt, Hh and Notch have emerged as key regulators of stem cell. It is apparent that some of these pathways are involved in shaping and maintaining the stem cell niche and therefore act as indirect regulators of the stem cell; other signalling pathways act as direct regulators of the stem cell.

Understanding how airway stem cells are regulated within their stem cell niche is not an easy task. Pulmonary progenitor cells, which express stem cell markers and possess the capacity to self-renew, can undergo oncogenic transformation [22, 23]. More specifically, the upregulation of Wnt/ß-catenin, Hedgehog (Hh), EGF and/or Notch pathways seems to represent a critical event that might be implicated in the initiation and development of some aggressive lung cancer types.

Role of Wnt pathway in stem cells

Wnt are secreted factors [24] that regulate cell growth, motility and differentiation during embryonic development. Members of the Wnt family have distinct expression patterns in embryo and adult organisms. Wnt-5a is involved in distal lung morphogenesis [25] and disruption of Wnt-5a signalling results in truncation of the trachea and abnormalities in distal lung architecture; absence of Wnt-5a increases lung cellular proliferation but does not interfere with differentiation of highly specialised lung epithelial cells [26].

Wnt acts by activating diverse signalling cascades inside cancer cells and has received attention from cancer researchers because many of its components play important roles in tumour formation.

Wnt signalling diversifies into three main branches.

– *The classical, also called canonical*, activates target genes through stabilisation of ß-catenin in the nucleus. The function of this pathway during embryonic development was originally elucidated by experimental analysis of axis development.

– *The planar cell polarity* regulates the polarity of cells through effects on their cytoskeletal organisation.

– *The Wnt/Ca*$^{2+}$ involves an activation of Ca^{2+}-sensitive signalling components through an increase in intracellular Ca^{2+} and can counteract the canonical Wnt pathway.

Role of Hh pathway in stem cells

During embryonic development, the Hedgehog (Hh) signalling pathway regulates proliferation and differentiation in a time/position-dependent fashion [27] so that developing tissues reach their correct size with the appropriate cell types.

Hh exerts its effects by activating gene transcription in target cells expressing the hedgehog receptor Patched. This interaction initiates a complex intracellular signal transduction cascade that activates the transcription factor Gli, one of three mammalian Gli genes related to the segment polarity gene Cubitus interruptus.

Many reports have illustrated the capacity of Hh signalling to regulate stem cell fates [27].

At the molecular level, a number of different growth factors have been shown to be important for lung development, and these include Sonic Hh (Shh) throughout a complex interplay between mesenchymal and epithelial cells. Shh normally plays a key role in branching morphogenesis, and acts as a mitogen for mesenchymal and possibly epithelial cells [28].

Role of Notch pathway in stem cells

Stem-cell maintenance, binary cell-fate decisions and induction of differentiation are the three main functions of Notch signalling in self-renewing tissues [29–34]. Notch

also has the ability to maintain stem/precursor cells in an undifferentiated state and this action is driven by hypoxia conditions. Actual data indicate that the stem cell regulatory Notch pathway shares in an interplay with the hypoxia response modulator HIF-1α to promote the onset of a stem/undifferentiated phenotype. These findings, linking stem cells with hypoxia survival, lead to the hypothesis that the control of stem cell survival and the regulation of hypoxia response are intimately coupled and that they share common control gene/pathways [35]. Hypoxia activates Notch-responsive promoters and increases expression of Notch direct downstream genes. The Notch intracellular domain interacts with HIF-1α, a global regulator of oxygen homeostasis, and HIF-1α is recruited to Notch-responsive promoters upon Notch activation under hypoxic conditions [36].

The stress response gene p66Shc (a mammalian longevity modulator) [37] is induced by exposure to hypoxic stimuli and it controls the expression of Notch3 (a stem cell regulatory gene) [38]. p66Shc/Notch3 interplay elicits an extracellular signal-regulatory kinase (ERK)-dependent upregulation of at least two genes: the Jagged-1 (a Notch ligand) [38] and CA-IX (the hypoxia-survival gene carbonic anhydrase) [39] inducing cell renewal of human stem progenitor cells [40].

Both the Notch receptor and its ligands are transmembrane proteins with large extracellular domains that consist primarily of EGF-like repeats followed by three cysteine-rich Notch/Lin12 repeats (LN). The amino-terminal EGF-like repeats participate in ligand binding, whereas the LN repeats prevent signalling in the absence of ligands.

The Notch receptors are activated by ligands presented by adjoining cells. Notch signalling is initiated by a receptor–ligand interaction between two neighbouring cells, which leads to two successive proteolytic cleavages that liberate the cytoplasmic domain of Notch (Notch-ICD) from the membrane [41]. Notch-ICD, with intrinsic nuclear localisation potential, is associated with the latent transcriptional activator CBS-1 [42–44].

Lung cancer stem cells

Many studies have indicated that several human cancer types, including lung, might arise from the malignant transformation of stem cells and their progenitors into cancer progenitor cells [11, 22]. Somatic genomic alterations (i.e., mutations, deletions, amplifications, chromosomal rearrangements) and change in DNA methylation might result in aberrant activation of distinct developmental cascades in adult stem cells and/or TA cells. These cancer progenitor cells, in turn, may subsequently give rise to a heterogeneous population of cancer cells.

Genetic alterations and/or sustained activation of distinct developmental mitogenic cascades occurring in a minority of adult stem cells and their progenitors might also lead to their oncogenic transformation [11]. This implicates the activation of numerous tumorigenic cascades that are mediated through distinct growth factor signalling pathways and that assume a critical role for the growth and survival cancer cells. The aberrant expression and/or activity of Wnt/ß-catenin, Shh/Smo, Notch and TGF-α/EGFR pathways seem to represent a critical event that might be implicated in the initiation and development of aggressive lung cancer types.

Wnt pathway in lung cancer

Molecular perturbations in specific components of the Wnt signalling pathway have been documented as transforming events in human lung cancer. Wnt signalling has been reported to be involved in lung carcinogenesis [45–47], and interestingly in lung cancer cell lines, through active canonical Wnt signalling [48] and also through Dishevelled (Dvl) overexpression in NSCLC.

Analysis of freshly resected tumours and lung cancer cell lines demonstrate that Dvl-3, a critical mediator of Wnt signalling, is overexpressed [49]. Specifically, Dvl-3 was overexpressed significantly in 75% of fresh NSCLC microdissected samples compared to control paired matched normal lung samples. NSCLC tumours with Dvl-3 expression showed higher expression of Wnt-1 or Wnt-2 compared to matched normal tissues.

The hypothesis that Wnt-2 overexpression may be involved in human lung carcinogenesis was assessed when freshly resected NSCLC tissues had increased expression of Wnt-2 protein compared with autologous-matched normal lung tissue controls. Furthermore, Wnt-2 expression was not observed in the small airway epithelial cells [50].

Wnt-5a expression is frequently upregulated in human cancers and may be involved in tumour development and progression [51].

Overexpression of Wnt-5a was associated with tumour cell proliferation in NSCLCs. Wnt-5a expression in squamous cell carcinomas was observed to be significantly higher than that in adenocarcinomas. Overexpression of Wnt-5a was associated with a poor prognosis, especially in squamous cell carcinomas, and in stage II to III (locally advanced) [52]. Regarding tumour biology, overexpression of Wnt-5a was associated with higher ki-67 proliferation indexes.

Furthermore, intratumoral Wnt-5a overexpression was also found to be associated with expression of ß-catenin and VEGF-A in stromal cells, which suggests the existence of a tumour–stromal interaction [53].

As a counterpart, Wnt-7a has been described as a tumour-suppressor gene. Expression of Wnt-7a is downregulated in most cancer cell lines and tumour samples. Analysis of multiple Wnt mRNAs in NSCLC cell lines and primary lung tumours revealed markedly decreased Wnt-7a expression when compared to normal short-term bronchial epithelial cell lines and normal uninvolved lung tissues [54]. These results indicate that loss of Wnt-7a expression

is a frequent molecular event that accompanies oncogenesis in the lung.

Hh pathway in lung cancer

Recent studies have illustrated the capacity of Hh signalling to regulate stem cell fates [55, 56]. Moreover, Hh pathway activation is essential for the growth, suggesting that the specification of progenitor cell fates by Hh signalling occupies a critical *master-regulator* role in controlling the malignant behaviour of cells [57, 58].

Inactivating mutations in Ptch result in aberrant Hh pathway activation and are associated with malignancy. In this setting, it has been shown that small-cell lung carcinoma is dependent on activation of Hedgehog (Hh) signalling. Small-cell lung carcinoma presents paracrine Hh signalling activity through the expression of the ligand, the receptor Ptch and transcriptional effector Gli. Strikingly, cells within SCLC *in vivo* demonstrate apparent compartmental segregation of cells which send and receive the Shh signal in an apparent duplication of the process seen in airway development and repair [59, 60].

Notch in lung cancer

Notch signalling is involved in both normal pulmonary and lung tumour development [61]. During human foetal development, Notch signalling controls pulmonary epithelial cell fate by activating Hairy/Enhancer of Split (HES) genes which in turn suppress genes required for neuroendocrine differentiation, such Achaete-Scute Homologue-1 (ASH-1) [61, 62]. PNECs selectively express ASH-1, which is required for the neuroendocrine features of these cells [63, 64]. Forced expression of ASH-1 results in lung hyperplasia and metaplasia of PNEC [65].

In varying cellular contexts, Notch can behave as an oncogene or a tumour suppressor [66], although Notch signalling is mainly linked to oncogenicity [67]. Notch contributes to the process of oncogenesis, working with oncoproteins able to override the G1-S checkpoint, providing complementary oncogenic features such as differentiation or resistance to apoptosis.

Although data regarding the role of the Notch pathway in human lung cancer are still limited, Notch function in lung cancer exhibits properties suggesting both tumour promotion and inhibition depending on the tumour cell type. In contrast to NSCLC, where Notch is suspected to have a growth promoting function, SCLC appears to be growth inhibited.

Among different Notch family receptors, Notch 1 and Notch 2 proteins are frequently expressed in NSCLC. Notch 3 seems to be overexpressed in 30–40% of NSCLC [68] and its expression is positively correlated with EGF receptor expression. A link between the Notch 3 and the epidermal growth factor receptor (EGFR) was established [69]. This interaction has also been observed in many mammalian developmental models [70–73]. Furthermore, aberrant expression of activated Notch 3 in the developing lungs of transgenic animals inhibits terminal differentiation of the maturing pneumocytes, supporting the hypothesis that Notch 3 may have a role in preventing normal terminal differentiation during oncogenesis [74] and the deregulation of Notch 3 plays a role in the transformation or maintenance of the neoplastic phenotype.

Although the canonical Notch pathway involves signalling through HES and related genes, Notch receptors interact with other signalling pathways, including the EGF and the mitogen-activated protein kinase (MAPK) pathway [75–77]. Complex crosstalk between the Ras and Notch has been extensively shown in cell-fate determination, moreover the Notch and Ras pathway play prominent roles in tumor growth and survival [78–82].

Also, Notch mRNA expression was detected in 7 of 25 NSCLC cell lines. It is suggested that Notch 3 may be an oncogene in non-small-cell carcinoma. Aberrant Notch 3 expression was found to be associated with a translocation in human lung cancer, as well as overexpressed in lung cancer cell lines. Expression of Notch 3 by immunohistochemistry using micro tissue-arrays (MTA) was found in 37% of adenocarcinoma, 45% of squamous cell carcinoma, 36% of large cell carcinoma, 25% of small cell carcinoma and 20% of carcinoid tumours. Moreover, a high percentage of lung cancer lines expressed Notch receptors (Jagged-1) and their transcriptional target genes (HES-1, Hey 1), suggesting that the Notch pathway plays an important role in lung cancer biology. The frequency of expression of Notch family receptors in lung cancer was assessed in a lung cancer line panel showing that Notch 1 was expressed in 14%, Notch 2 in 62%, Notch 3 in 41% and Notch 4 in 10% of cell lines analysed [83]. Other investigators observed high expression of the Hey 1 and Hey L in lung cancer tumorigenesis [61].

Conclusion

Advances in the molecular biology of stem cells and lung cancer will offer new opportunities for the design of new targeted drugs. The dependency of lung cancer development on the spread of stem cells across the respiratory system seems to be congruent and several lines of basic and clinical investigation are being launched. Proof-of-concept trials are currently recruiting patients in this setting administering anti-stem cell drugs alone or in combination with classical chemotherapy. There is a need to find new biomarkers that allow selection of the most suitable patients to receive anti-stem cell drugs.

Conflict of interest The authors declare that they have no conflict of interest relating to the publication of this manuscript.

References

1. Jemal A, Siegel R, Xu J et al (2010) Cancer statistics, 2010. CA Cancer J Clin 60:277–300
2. Langer C, Soria JC (2010) The role of anti-epidermal growth factor receptor and anti-vascular endothelial growth factor therapies in the treatment of non-small-cell lung cancer. Clin Lung Cancer 11:82–90
3. Kwak EL, Bang YJ, Camidge DR (2010) Anaplastic lymphoma kinase inhibition in non–small cell lung cancer. N Engl J Med 363:1693–1703
4. Takebe N, Ivy SP (2010) Controversies in cancer stem cells: targeting embryonic signaling pathways. Clin Cancer Res 16:3106–3112
5. Cardoso W (2000) Lung morphogenesis revisited: old facts, current ideas. Dev Dyn 219:121–130
6. Cardoso W (2001) Molecular regulation of lung development. Ann Rev Physiol 63:471–494
7. Crapo JD, Barry BE, Gehr P et al (1982) Cell numbers and cell characteristics of the nor-mal human lung. Am Rev Resp Dis 125:332–337
8. Evans MJ, Shami SG (1989) Lung cell kinetics. In: Massaro D (ed) Lung cell biology, vol. 41. Marcel Dekker, New York, pp 1–36
9. Jetten AM (1997) Growth and differentiation factors in tracheobronchial epithelium. Am J Physiol Lung Cell Mol Physiol 260:L361–L373
10. Shami SG, Evans MJ (1992) Kinetics of pulmonary cells. In: Parent RA (ed) Treatise on pulmonary toxicology. Comparative biology of the normal lung. CRC Press, Boca Raton, FL, pp 93–108
11. Kim CF, Jackson EL, Woolfender AE et al (2005) Identification of bronchioalveolar stem cells in normal lung and lung cancer. Cell 121:823–835
12. Liu X, Driskell RR, Engelhardt JF (2004) Airway glandular development and stem cells. Curr Top Dev Biol 64:33–56
13. Griffiths MJ, Bonnet D, Janes SM (2005) Stem cells of the alveolar epithelium. Lancet 366:249–260
14. Mason RJ, Williams MC, Moses HL et al (1997) Stem cells in lung development, disease, and therapy. Am J Respir Cell Mol Biol 16:355–363
15. Borthwick DW, Shahbazian M, Krautz QT et al (2001) Evidence for stem cell niches in the tracheal epithelium. Am J Respir Mol Biol 29:662–670
16. Engelhardt JF (2001) Stem cell niches in the mouse airway. Am J Respir Mol Biol 24:649–652
17. Engelhardt JF, Schlossberg H, Yankaskas JR et al (1995) Progenitor cells of the adult human airway involoved in submucosal gland development. Development 121:2031–2046
18. Engelhardt JF, Allen E, Wilson JM (1991) Reconstitution of tracheal grafts with a genetically modified epithelium. Proc Natl Acad Sci U S A 88:11192–11196
19. Reynolds SD, Giangreco A, Power JHT et al (2000) Neuroepithelial bodies of pulmonary airways serve as a reservoir of progenitor cells capable of epithelial regeneration. Am J Pathol 156:269–278
20. Hoyt RF, Sorkin SP, McDowell EM et al (1993) Neuroepithelial bodies and growth of the airway epithelium in developing hamster lung. Anat Rec 236:15–22
21. Hong KV, Reynolds SD, Giangreco A et al (2001) Clara cell secretory protein-expressing cells of the airway neuroepithelial body microenvironment after progenitor cells depletion. Am J Resp Cell Mol. 24:670–682
22. Berns A (2005) Stem cells for lung cancer? Cell 121:811–813
23. Beachy PA, Karhadkar SS, Berman DM (2004) Tissue repair and stem cell renewal in carcinogenesis. Nature 432:324–331
24. Miller JR (2002) The Wnts. Genome Biol 3:S3001–S3015
25. Gavin BJ, McMahon JA, McMahon AP (1990) Expression of multiple novel Wnt-1/int-1 related genes during fetal and adult mouse development. Genes Dev 4:2319–2332
26. Li C, Xiao J, Hormi K et al (2002) Wnt5a participates in distal lung morphogenesis. Dev Biol 248:68–81
27. Ingham PW, McMahon AP (2001) Hedgehog signaling in animal development: paradigms and principles. Genes Dev 15:3059–3087
28. Bellusci S, Furuta Y, Rush MG et al (1997) Involvement of Sonic hedgehog (Shh) in mouse embryonic lung growth and morphogenesis. Development 124:53–63
29. Rangarajan A, Talora C, Okuyama R et al (2001) Notch signaling is a direct determinant of keratinocyte growth arrest and entry into differentiation. EMBOJ 20:3427–3436
30. Varnum-Finney B, Purton LE, Yu M et al (1998) The Notch ligand, Jagged-1, influences the development of primitive hematopoietic precursor cells. Blood 91:4084–4091
31. Jones P, May G, Healy L et al (1998) Stromal expression of Jagged 1 promotes colony formation by fetal hematopoietic progenitor cells. Blood 92:1505–1511
32. Kimble J, Simpson P (1997) The LIN-12/Notch signaling pathway and its regulation. Annu Rev Cell Dev Biol 13:333–361
33. Artavanis-Tsakonas S, Matsuno K, Fortini ME (1995) Notch signaling. Science 268:225–232
34. Lowell S, Jones P, Le Roux I et al (2000) Stimulation of human epidermal differentiation by delta-notch signaling at the boundaries of stem-cell clusters. Curr Biol 10:491–500
35. Cejuto-Martin P, Johnson RS (2005) A new Notch in the HIF belt: how hypoxia impacts differentiation. Dev Cell 9:575–576
36. Sansone P, Storci G, Giovanni C et al (2007) P66Shc/Notch-3 interplay controls self-renewal and hypoxia survival in human stem/progenitor cells of the mammary gland expanded in vitro as mammospheres. Stem Cells 25:807–815
37. Migliaccio E, Giorgio M, Mele S et al (1999) The p66Shc adaptor protein controls oxidative stress response and life span in mammals. Nature 402:309–313
38. Bray SJ (2006) Notch signalling: a simple pathway becomes complex. Nat Rev Mol Cell Biol 7:678–689
39. Robertson N, Potter C, Harris AL (2004) Role of carbonic anhydrase IX in human tumor cell growth, survival, and invasion. Cancer Res 64:6160–6165
40. Dontu G, Jackson KW, McNicholas E et al (2004) Role of Notch signaling in cell-fate determination of human mammary stem/progenitor cells. Breast Cancer Res 6:R605–R615
41. Baron M (2003) An overview of the Notch signaling pathway. Semin Cell Dev Biol 14:113–119
42. Kao HY, Ordentlich P, Koyano-Nakagawa N et al (1996) A histone deacetylase corepressor complex regulates the Notch signal transduction pathway. Genes Dev 12:2269–2277
43. Hsieh JJ, Zhou S, Chen L et al (1999) CIR, a corepressor linking the DNA binding factor CBF1 to the histone deacetylase complex. Proc Natl Acad Sci U S A 96:23–28
44. Morel V, Lecourtois M, Massiani O et al (2001) Transcriptional repression by suppressor of hairless involves the binding of a hairless-dCtBP complex in *Drosophila*. Curr Biol 11:789–792
45. Sunaga N, Kohno T, Kollings FT et al (2001) Constitutive activation of the Wnt signaling pathway by CTNNB1 (beta-catenin) mutations in a subset of human lung adenocarcinoma. Genes Chromosomes Cancer 30:316–321
46. Ueda M, Gemmill RM, West J et al (2001) Mutations of the beta- and gamma-catenin genes are uncommon in human lung, breast, kidney, cervical and ovarian carcinomas. Br J Cancer 85:64–68
47. Hommura F, Furuuchi K, Yaamazaki K et al (2002) Increased expression of beta-catenin predicts better prognosis in non-small cell lung carcinomas. Cancer 94:752–758
48. Winn RA, Bremmes RM, Bemis L et al (2002) gamma-Catenin expression is reduced or absent in a subset of human lung cancers and re-expression inhibits transformed cell growth. Oncogene 21:7497–7506
49. Uematsu K, He B, You L et al (2003) Activation of the Wnt pathway in non small cell lung cancer: evidence of dishvelled overexpression. Oncogene 22:7218–7221
50. You L, He B, Xu Z et al (2004) Inhibition of Wnt-2-mediated signaling induces programmed cell death in non-small-cell lung cancer cells. Oncogene 23:6170–6147
51. Lejeune S, Huguet EL, Hamby A et al (1995) Wnt5a cloning, expression, and up-regulation in human primary breast cancers. Clin Cancer Res 1:215–222
52. Taki M, Kamata N, Yokoyama K et al (2003) Down-regulation of Wnt-4 and up-regulation of Wnt-5a expression by epithelial-mesenchymal transition in human squamous carcinoma cells. Cancer Sci 94:593–597
53. Huang C, Liu D, Nakano J et al (2005) Wnt5a expression is associated with the tumor proliferation and the stromal vascular endothelial growth factor. An expression in non-small cell lung cancer. J Clin Oncol 23:8765–8773
54. Calvo R, West J, Franklin W et al (2000) Altered HOX and WNT7A expression in human lung cancer. Proc Natl Acad Sci U S A 97:12776–12781
55. Bale AE, Yu KP (2001) The hedgehog pathway and basal cell carcinomas. Hum Mol Genet 10:757–762
56. Ingham PW, McMahon AP (2001) Hedgehog signaling in animal development paradigms and principles. Genes Dev 15:3059–3087
57. Berman DM, Karhadkar SS, Hallahan AR et al (2002) Medulloblastoma growth inhibition by hedgehog pathway blockade. Science 297:1559–1561
58. Kalderon D (2000) Transducing the hedgehog signal. Cell 103:371–374
59. Watkins DN, Berman DM, Burkholder SG et al (2003) Hedgehog signaling within airway epithelial progenitors and in small-cell lung cancer. Nature 422:313–317
60. Watkins DN, Berman DM, Baylin SB (2003) Hedgehog signaling progenitor phenotype in small-cell lung cancer. Cell Cycle 2:196–198
61. Collins BJ, Kleeberger W, Ball DW (2004) Notch in lung development and lung cancer. Semin Cancer Biol 14:357–364
62. Ito T, Udaka N, Yazawa T et al (2000) Basic helix-loop-helix transcription factors regulate the neuroendocrine differentiation of fetal mouse pulmonary epithelium. Development 127:3913–3921
63. Borges M, Linnoila RI, van de Velde HJ et al (1997) An achaete-scute homologue essential for neuroendocrine differentiation in the lung. Nature 386:852–855
64. Johnson JE, Birren SJ, Saito T et al (1992) DNA binding and transcriptional regulatory activity of mammalian achaete-scute homologous (MASH) proteins revealed by interaction with a muscle-specific enhancer. Proc Natl Acad Sci USA 89:3596–3600
65. Linnoila RJ, Zhao B, DeMayo JL et al (2000) Constitutive achaete-scute homologue-1 promotes airway dysplasia and lung neuroendocrine tumors in transgenic mice. Cancer Res 60:4005–4009
66. Radtke F, Raj K (2003) The role of Notch in tumorigenesis: oncogene or tumour suppressor? Nat Rev Cancer 3:756–767
67. Miele I, Golde T, Osborne B (2006) Notch signaling in cancer. Curr Mol Med 6:905–918
68. Haruki N, Kawaguchi KS, Eichenberg S et al (2005) Dominant-negative Notch3 receptor inhibits mitogen-activated protein kinase pathway and the growth of human lung cancers. Cancer Res 65:3555–3561
69. Dang PT, Gazdar AF, Virmani AK et al (2000) Chromosome 19 translocation, overexpression of Notch3, and human lung cancer. J Natl Cancer Inst 92:1355–1357
70. Faux CH, Turnley AM, Epa R et al (2001) Interactions between fibroblast growth factors and

Notch regulate neuronal differentiation. J Neurosci 21:5587–5596
71. Mustanen T, Tummen M, Mikani T et al (2002) Lunatic fringe, FGF and BMP regulate the Notch pathway during epithelial morphogenesis of teeth. Dev Biol 248:281–293
72. Weinstein BM, Lawson ND (2002) Arteries, veins, Notch, and VEGF. Cold Spring Harb Symp Quant Biol 67:155–162
73. Hart A, Papadopoulus S, Edlund H (2003) Fgf10 maintains notch activation, stimulates proliferation, and blocks differentiation of pancreatic epithelial cells. Dev Dyn 228:185–193
74. Dang TP, Eichenberger S, Gonzalez A et al (2003) Constitutive activation of Notch 3 inhibits terminal epithelial differentiation in lungs of transgenic mice. Oncogene 22:1988–1997
75. Miyamoto Y, Maitra A, Ghosh B et al (2003) Notch mediates TGFα-induced changes in epithelial differentiation during pancreatic tumorigenesis. Cancer Cell 3:565–576
76. Patel NS, Li JL, Generali D et al (2005) Up-regulation of delta-like 4 ligand in human tumor vasculature and the role of basal expression in endothelial cell function. Cancer Res 65:8690–8697
77. Yao AS, Bais C, Greenwald I (2004) Crosstalk between the EGFR and LIN-12/Notch pathway in *C. elegans* vulval development. Science 303:663–666
78. Weijzen S, Rizzo P, Braid M et al (2002) Activation of Notch-1 signaling maintains the neoplastic phenotype in human Ras-transformed cells. Nat Med 8:979–986
79. Carmena A, Buff E, Halfon MS et al (2002) Reciprocal regulatory interactions between the Notch and Ras signaling pathways in the *Drosophil*a embryonic mesoderm. Dev Biol 244:226–242
80. Berset T, Hoier EF, Battu G et al (2001) Notch inhibition of Ras signaling through MAP kinase phosphatase LIP-1 during *C. elegans* vulval development. Science 291:1055–1058
81. Culi J, Martin-Blanco E, Modolell J (2001) The EGF receptor and N signalling pathways act antagonistically in *Drosophila* mesothorax bristl patterning. Development 128:299–308
82. Fitzgerald K, Harrington A, Leder P (2000) Ras pathway signals are required for notch-mediated oncogenesis. Oncogene 19:4191–4198
83. Konishi J, Kawaguchi KS, Vo H et al (2007) γ-secretase inhibitor prevents Notch 3 activationb and reduces proliferation in human lung cancers. Cancer Res 67:8051–8057