Year-in-Review of Lung Cancer

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In the last several years, we have made slow but steady progress in understanding molecular biology of lung cancer. This review is focused on advances in understanding the biology of lung cancer that have led to proof of concept studies on new therapeutic approaches. The three selected topics include genetics, epigenetics and non-coding RNA. This new information represents progress in the integration of molecular mechanisms that to identify more effective ways to target lung cancer.

Key Words: Lung Neoplasms; Molecular Biology; Genetics; Epigenomics; RNA, Untranslated

Introduction

Lung cancer is the leading cause of cancer-related mortality in the developed world, accounting for 26~29% of all cancer deaths[1]. Non-small cell lung cancer (NSCLC) accounts for 80% of all lung cancer diagnosis and the majority (60~80%) of patients are diagnosed at an advanced stage. Consequently, the prognosis for NSCLC remains poor, with a 5-year survival rate of 15%[2].

The histologic subtype of lung cancer has had little impact on the selection of therapy. Non-squamous cell histology is an indication for chemotherapy such as pemetrexed and bevacizumab. This type of stratification represents the first step toward personalized therapy in NSCLC[3]. Beyond histology, some molecular alterations have been shown to correlate with response to treatment with chemotherapy. New therapies targeting the *EML4-ALK, BRAF, FGFR* and other molecular alterations are under way to help define specific subsets of patients responsive to certain molecularly targeted treatments[4].

The ongoing identification of novel molecular abnormalities in lung cancer continues to present exciting opportunities in targeted therapy. In this brief review, there will be an effort to discuss some of the developments in lung cancer biology. As an in depth review of all areas of biology is not possible within this manuscript, three fields (genetics, epigenetics and non-coding RNA) were selected for detailed review (Figure 1).

Literature Search

A literature search from January to December 2011 was conducted using PubMed and the following search terms: "Non-small cell lung cancer/molecular biology," "Non-small cell lung cancer/genetics," "Non-small cell lung cancer/epigenetics" and "Lung cancer/non-coding RNA." Conference proceedings in 2011 for American Society of Clinical Oncology (ASCO), American Association for Cancer Research (AACR), and the International Society for the Study of Lung Cancer (IASLC) World Lung Congresses were searched.

Genetics

Analysis of cancer genome sequences using DNA microarrays and capillary-based DNA sequencing provide

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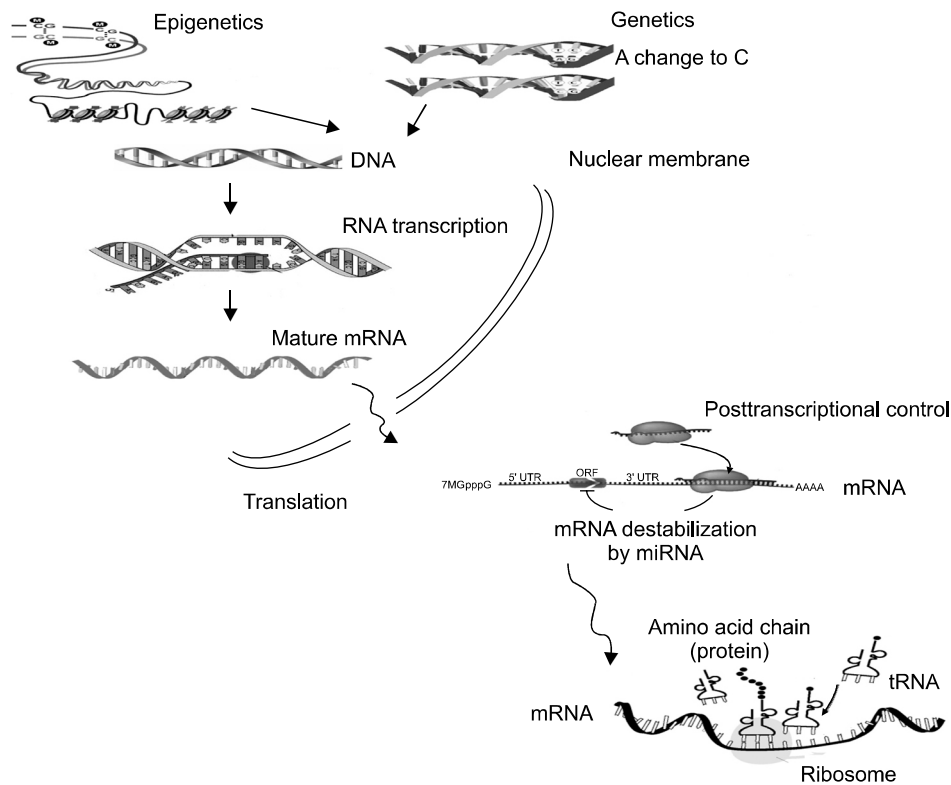E-mail: sk1609@hanmail.net

Figure 1. Gene expression via genetic, epigenetic and posttranscriptional modification is a key regulatory process in the cell.

insights for understanding cancer biology, diagnosis and therapy. However, there are particular challenges for the detection and diagnosis of cancer genome alterations. For example, some genomic alterations in cancer are prevalent at a low frequency in clinical samples, often owing to substantial admixture with non-malignant cells. Next-generation sequencing technologies can solve such problems. Furthermore, these new sequencing methods make it feasible to discover novel chromosomal rearrangements and microbial infections, and to resolve copy number alterations at very high resolution[5].

Several recent studies using next generation sequencing technologies have been reported in lung cancer. Lipson et al.[6] analyzed genomic DNA from 24 NSCLC formalin-fixed paraffin-embedded specimens using an assay that captured and sequenced 2,574 coding exons representing 145 cancer-relevant genes (genes associated with cancer-related pathways, targeted therapy or prognosis) plus 37 introns from 14 genes that are frequently rearranged in cancer. Among the 24 NSCLCs, 50 alterations were identified in 21 genes, with at least

one alteration being present in 83% (20 out of 24) of the tumors. Twelve genes were altered in multiple tumors. In addition to the known NSCLC gene alterations, G1849T (V617F) *JAK2* mutation and gene fusion joining exons 1~15 of *KIF5B* to exons 12~20 of *RET* were notable discoveries. *KIF5B-RET* fusion was also reported in lung adenocarcinoma of Korean patients using next-generation sequencing technologies[7]. These findings suggest that RET kinase inhibitors and JAK inhibitors should be tested in prospective clinical trials for therapeutic benefit in individuals with NSCLC that carry these genomic alterations. In another novel finding using next generation sequencing technique, Jung et al.[8] analyzed the transcriptome of the NSCLC cell line H2228 and discovered a fusion transcript composed of multiple exons of *ALK* and *PTPN3*. Detailed analysis of the genomic structure revealed that a portion of genomic region encompassing exons 10 and 11 of *ALK* had been translocated into the intronic region between exons 2 and 3 of *PTPN3*[8]. In lung cancer, five fusion partners of ALK have been reported *EML4, TFG, KIF5B, KLC1* and *PTPN3*[9]. As more *ALK* fusion partners are identi-

fied, the population of patients with *ALK* fusion who may potentially benefit from ALK inhibitor therapy may be extended.

The next-generation sequencing could provide innovative information in cancer biology. But, to be widely and routinely used in clinical practice, there needs to be further reduction in cost, flexibility in throughput and efficiency of data generation, analysis and management.

Epigenetics

Epigenetics is defined as heritable changes in gene expression that are not attributable to changes in the sequence of DNA. Epigenetic regulation of gene expression has emerged as a fundamental pathway in the pathogenesis of lung cancer. The predominant epigenetic mechanisms are DNA methylation and histone modifications[10-12]. DNA methylation is the covalent addition or subtraction of a methyl group to a cytosine nucleotide in a sequence of DNA. Methylation is controlled by a family of specific enzymes known as DNA methyltransferases (DNMTs). The addition of methyl groups can be highly specific to a particular gene. Hypermethylation of CpG islands in the promoter region of a gene can result in transcriptional silencing of the gene, and subsequent loss of protein expression[13]. The molecular mechanism of demethylated DNA has long been unknown. The recent discovery that the three members of the ten-eleven translocation (TET) protein family can convert 5-methylcytosine (5-mC) into 5-hydroxymethylcytosine (5-hmC) has provided a potential mechanism leading to DNA demethylation (Figure 2). Moreover, the demonstration that *TET2* is frequently mutated in hematopoietic tumors suggests that the TET proteins could play a crucial role in tumorigenesis[14]. The levels of 5-hmC of lung cancer tissues have been shown to be markedly depleted with up to a 5-fold reduction compared with normal lung tissue[15]. The biological properties of DNA demethylation by TET proteins in solid tumors was reported earlier this year. Kudo et al.[16] reported that down-regulation of *TET1* and loss of 5-hmC were induced by oncogene-dependent cellular transformation. These results suggest critical roles of aberrant DNA demethylation for oncogenic processes in solid tissues.

The discovery of 5-hmC and *TET* in lung cancer has added a new and potentially important dimension to our perception of DNA methylation. Further studies are required to confirm role of 5-hmc and *TET* and to investigate potential clinical applications in lung cancer.

Non-coding RNAs (ncRNA)

A ncRNA is a functional RNA molecule that is not translated into a protein. ncRNAs are regarded as regulators of cell cycle progression, proliferation, and fate. There are numerous classes of ncRNAs, including microRNAs (miRNA, ～22 nt) and Piwi-interacting RNAs (piRNAs, 18～30 nt), short translational-regulatory RNAs (100～200 nt), and much longer ncRNAs (lncRNAs, up to 10,000 nt)[17,18]. So far, the function of miRNAs as key components of the RNA interference (RNAi) pathway, their role as tumor suppressors, and their impact on tu-
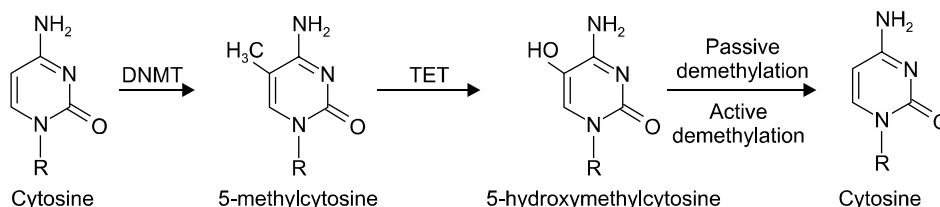

Figure 2. Enzymatic conversion of cytosine to 5-hydroxymethylcytosine. DNA methyltransferases (DNMTs) convert cytosine to 5-methylcytosine. 5-hydroxymethylcytosine be converted to 5-hydroxymethylcytosine in an enzymatic process involving members of the ten-eleven translocation (TET) protein family. 5-hydroxymethylcytosine could be converted to cytosine through a passive demethylation or an active demethylation pathway.
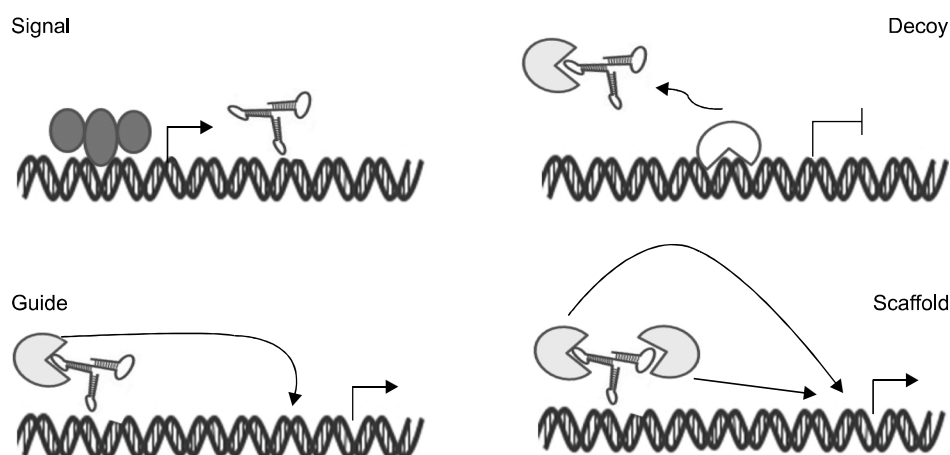
Figure 3. Schematic diagram of the four archetypes of longer ncRNAs (lncRNA) mechanism. Signaling: LncRNA expression can be stimulated in response to certain stimuli, such as cellular stress and temperature. Decoys: Specific lncRNAs are transcribed and then bind to and titrate away protein factors. Guides: LncRNAs can be molecular guides by localizing particular ribonucleoprotein complexes to specific chromatin targets. Scaffolds: LncRNAs can bring together multiple proteins to form ribonucleoprotein complexes. Adopted from Wang and Chang[19]. Mol Cell 2011;43:904-14.

morigenesis have been demonstrated. Nevertheless, little is known about lncRNAs and their impact on tumorigenesis regulatory processes. LncRNAs participate in a wide-range of biological processes. Almost every step in the life cycle of genes can be influenced by lncRNAs through posttranscriptional gene regulation, RNA maturation, RNA transport and transcriptional gene silencing. The molecular mechanisms of lncRNAs are classified into four archetypes: signals, decoys, guides and scaffolds (Figure 3). Several lncRNAs possess characteristics from multiple archetypes that, in combination, are critical to their eventual biological function[19]. Metastasis-associated-in-lung-adenocarcinoma-transcript-1 (MALAT-1) is an lncRNA and highly expressed in several tumor types. MALAT-1 strongly associates with serine-arginine rich splicing factor (SR) proteins involved in both constitutive and alternative splicing, and the levels of MALAT-1 regulate the cellular levels of phosphorylated SR proteins. These findings imply that the MALAT-1 may serve a function in the regulation of alternate splicing by modulating the activity of SR proteins[20]. MALAT-1 is highly expressed in several human NSCLCs and is a strong regulator of NSCLC migration and invasion. MALAT-1 RNA expression in squamous cell carcinoma of the lung has been associated with a poor prognosis[21].

miRNAs regulate gene expression at the post-transcriptional level through sequence-specific interactions with 3'-untranslated regions in mRNAs and also via translation inhibition or degradation of mRNAs. Recent studies have demonstrated that epigenetic mechanisms regulate the expression of miRNAs. An extensive analysis of genomic sequences of miRNA genes has shown that approximately half are associated with CpG islands. Therefore three main epigenetic events including the aberrant hypermethylation of tumor suppressor genes, global DNA hypomethylation and post-translational modifications of histone could affect miRNA expression as well[22]. Some miRNAs are up-regulated upon the exposure of cells to the agent 5-aza-2'-deoxycytidine, upon mutation of DNMTs or upon treatment with histone deacetylase inhibitors[23-25]. Conversely, another subset of miRNAs controls the expression of important epigenetic regulators including DNA methyltransferases, histone deacetylases and polycomb group genes[26-28]. This complicated network of feedback between miRNAs and epigenetic pathways appears to form an epigenetics-miRNA regulatory circuit, which may organize the whole gene expression profile in lung cancer (Figure 4).

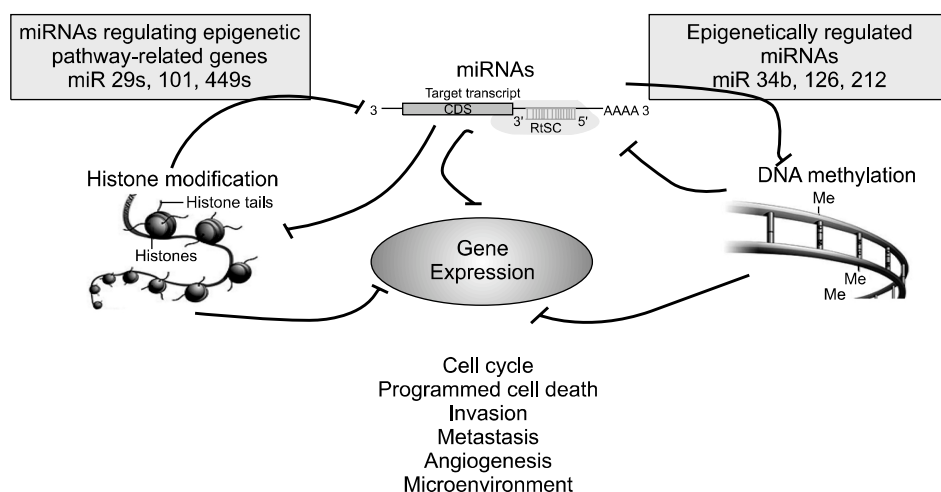It has become evident in recent years that the de-reg-

ulation of miRNAs and lncRNAs plays a critical role in malignant transformation and tumor cell behavior. The functional role for the vast majority of these ncRNA is still in question. Further study of ncRNA could yield new RNA-based targets for the prevention and treatment of lung cancer.

Conclusions

Lung cancer is a disease of genetic alteration, epigenetic changes as well as transcription control by non-coding RNA. The biology of lung cancer may also predict response or outcome to certain chemotherapeutic agents, serving as biomarkers that could inform clinical decisions. Along with newly discovered tumor biologic mechanisms, these findings raise hope that cancer can and will be vanquished.

Acknowledgements

References

1. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin 2010;60:277-300.
2. Spiro SG, Silvestri GA. One hundred years of lung cancer. Am J Respir Crit Care Med 2005;172:523-9.
3. Kulesza P, Ramchandran K, Patel JD. Emerging concepts in the pathology and molecular biology of advanced non-small cell lung cancer. Am J Clin Pathol 2011;136:228-38.
4. Govindan R. Summary of presentation from the targeted therapy in lung cancer meeting. J Thorac Oncol 2011;6(11 Suppl 4):S1757.
5. Meyerson M, Gabriel S, Getz G. Advances in understanding cancer genomes through second-generation sequencing. Nat Rev Genet 2010;11:685-96.
6. Lipson D, Capelletti M, Yelensky R, Otto G, Parker A, Jarosz M, et al. Identification of new *ALK* and *RET* gene fusions from colorectal and lung cancer biopsies. Nat Med 2012;18:382-4.
7. Ju YS, Lee WC, Shin JY, Lee S, Bleazard T, Won JK, et al. A transforming *KIF5B* and *RET* gene fusion in lung adenocarcinoma revealed from whole-genome and transcriptome sequencing. Genome Res 2012;22: 436-45.
8. Jung Y, Kim P, Jung Y, Keum J, Kim SN, Choi YS, et al. Discovery of *ALK-PTPN3* gene fusion from human non-small cell lung carcinoma cell line using next generation RNA sequencing. Genes Chromosomes Cancer 2012;51:590-7.
9. Togashi Y, Soda M, Sakata S, Sugawara E, Hatano S, Asaka R, et al. *KLC1-ALK*: a novel fusion in lung cancer identified using a formalin-fixed paraffin-embedded tissue only. PLoS One 2012;7:e31323.
10. Jones PA, Baylin SB. The fundamental role of epigenetic events in cancer. Nat Rev Genet 2002;3:415-28.
11. Son JW, Kim YJ, Cho HM, Lee SY, Lee SM, Kang JK, et al. Promoter hypermethylation of the *CFTR* gene and clinical/pathological features associated with non-

small cell lung cancer. Respirology 2011;16:1203-9.

12. Gery S, Komatsu N, Kawamata N, Miller CW, Desmond J, Virk RK, et al. Epigenetic silencing of the candidate tumor suppressor gene Per1 in non-small cell lung cancer. Clin Cancer Res 2007;13:1399-404.

13. Hamilton JP. Epigenetics: principles and practice. Dig Dis 2011;29:130-5.

14. Williams K, Christensen J, Helin K. DNA methylation: TET proteins-guardians of CpG islands? EMBO Rep 2012;13:28-35.

15. Jin SG, Jiang Y, Qiu R, Rauch TA, Wang Y, Schackert G, et al. 5-Hydroxymethylcytosine is strongly depleted in human cancers but its levels do not correlate with *IDH1* mutations. Cancer Res 2011;71:7360-5.

16. Kudo Y, Tateishi K, Yamamoto K, Yamamoto S, Asaoka Y, Ijichi H, et al. Loss of 5-hydroxymethyl-cytosine is accompanied with malignant cellular transformation. Cancer Sci 2012;103:670-6.

17. Eddy SR. Non-coding RNA genes and the modern RNA world. Nat Rev Genet 2001;2:919-29.

18. ENCODE Project Consortium, Birney E, Stamatoyanno-poulos JA, Dutta A, Guigó R, Gingeras TR, et al. Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. Nature 2007;447:799-816.

19. Wang KC, Chang HY. Molecular mechanisms of long noncoding RNAs. Mol Cell 2011;43:904-14.

20. Gibb EA, Brown CJ, Lam WL. The functional role of long non-coding RNA in human carcinomas. Mol Cancer 2011;10:38.

21. Schmidt LH, Spieker T, Koschmieder S, Schäffers S, Humberg J, Jungen D, et al. The long noncoding MALAT-1 RNA indicates a poor prognosis in non-small cell lung cancer and induces migration and tumor growth. J Thorac Oncol 2011;6:1984-92.

22. Sato F, Tsuchiya S, Meltzer SJ, Shimizu K. MicroRNAs and epigenetics. FEBS J 2011;278:1598-609.

23. Watanabe K, Emoto N, Hamano E, Sunohara M, Kawakami M, Kage H, et al. Genome structure-based screening identified epigenetically silenced microRNA associated with invasiveness in non-small-cell lung cancer. Int J Cancer 2012;130:2580-90.

24. Incoronato M, Urso L, Portela A, Laukkanen MO, Soini Y, Quintavalle C, et al. Epigenetic regulation of miR-212 expression in lung cancer. PLoS One 2011;6: e27722.

25. Lujambio A, Ropero S, Ballestar E, Fraga MF, Cerrato C, Setién F, et al. Genetic unmasking of an epigeneti-cally silenced microRNA in human cancer cells. Cancer Res 2007;67:1424-9.

26. Zhang JG, Guo JF, Liu DL, Liu Q, Wang JJ. Micro-RNA-101 exerts tumor-suppressive functions in non-small cell lung cancer through directly targeting en-hancer of zeste homolog 2. J Thorac Oncol 2011;6: 671-8.

27. Jeon HS, Lee SY, Lee EJ, Yun SC, Cha EJ, Choi E, et al. Combining microRNA-449a/b with a HDAC in-hibitor has a synergistic effect on growth arrest in lung cancer. Lung Cancer 2012;76:171-6.

28. Fabbri M, Garzon R, Cimmino A, Liu Z, Zanesi N, Callegari E, et al. MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyl-transferases 3A and 3B. Proc Natl Acad Sci U S A 2007;104:15805-10.