

## Precision prevention: A focused response to shifting paradigms in healthcare

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### Impact statement

The study of LINE-1 retroelements and their role in the pathogenesis of diseases of the lung such as COPD and lung cancer may provide valuable diagnostic and therapeutic tools to identify pre-emptively individuals at risk of pulmonary disease progression. Limited information is presently available on the role of LINE-1 in the regulation of disease phenotypes and the development of novel therapeutics designed to curtail LINE-1-mediated pathogenesis. Successful implementation of precision prevention strategies may help to spare those impacted by obstructive pulmonary disease from continued deterioration, while realizing significant cost savings and improved quality of healthcare.

### Abstract

Human health and disease are defined at the intersection of molecules, environment, and lifestyle, which combined determine phenotypic outcomes. To date, most clinical applications in the precision medicine space have focused on DNA, with lesser attention given to the biology encoded by RNA molecules, or the complexity of biological regulation at the level of proteins and metabolites. The totality of this information must be integrated in ways that allow for implementation of knowledge-based health promotion and prevention strategies that can help address current limitations in healthcare delivery. This review describes recent advances in the development of diagnostic tools for early detection and stratification of individuals suffering from chronic obstructive pulmonary disease and their heightened risk for the development of lung malignancies.

**Keywords:** Precision medicine, precision prevention, healthcare, COPD, LINE-1, health promotion

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### Introduction: The evolution of healthcare from symptom-based models to preventative precision medicine

The practice of medicine has evolved from a symptom-based model of diagnosis and treatment to one that is increasingly dependent upon the integration of patterns of disease aided by artificial intelligence and computer algorithms.<sup>1</sup> This evolution has been operationalized in the form of evidence-based practice in ways that have not only defined standards of care, but also established the underpinnings of what is now recognized as precision medicine.<sup>2</sup> The pressures for evolutionary shift in healthcare practice are complex and include among others, non-sustainable health care costs, suboptimal efficiencies in clinical practice, technological advances, and creation

of interdisciplinary teams. Equally important has been a reframing of patient expectations, with increased emphasis on disease prevention.

The AMA published recently data obtained from the Centers for Medicare and Medicaid Services indicating that US spending on drugs will grow faster than any other health care service over the next decade.<sup>3</sup> The Office of Government indicated that US health care spending would grow faster than the overall economy, squeezing public insurance programs and employers who provide coverage.<sup>3</sup> In total, it has been predicted that US healthcare spending will climb 5.3% in 2018, reflecting rising prices of medical goods and services and higher Medicaid costs, a trend that will continue for the next decade. In light of these statistics, one of the key mandates of precision health care is a focus on disease prevention. Disease prevention relies on

the availability of screening approaches that can help identify those with increased susceptibility; however, at this time relatively few precision-based preventative approaches are implemented in clinical practice. Given the aforementioned pressures, concerted efforts to develop these assays and define their sensitivity and specificity remain a priority and will likely be instrumental in shaping our health care system over the next century.

## Advances in genome science usher in the era of precision medicine

Our current understanding of susceptibility is predicated upon the discovery that many non-communicable diseases originate from an individual's DNA 'hardware' or the epigenetic 'software' that controls its use and, by extension, nascent RNA molecules and proteins. Thus, advances in precision medicine are often hinged upon our understanding of genome sciences. Despite major technological advances, our conventional understanding of genetic functions applies to less than 2% of the genome, and much remains to be learned about the roles of non-coding regions.<sup>4</sup> Completion of the human genome project 15 years ago was followed by studies aiming to fill this knowledge gap, known as The Encyclopedia of DNA Elements (ENCODE). The ENCODE project used genome-wide datasets of transcription factor binding sites, histone modifications, DNase I hypersensitivity, 3D chromatin interactions, and other platforms, to begin to assign functionality to DNA sequence. Major ENCODE findings and their implications are summarized in Table 1, with perhaps the most important conclusion being that health, disease, and susceptibility are not simply explained by genetic polymorphisms, but involve complex interactions mediated by the epigenome and non-coding regions.<sup>5,6</sup> Furthermore, the epigenome is shaped by malleable factors such as diet, stress, life history, and social factors to emphasize the importance of the environment as an arbiter of health and

disease.<sup>7</sup> Environmental exposures are an integral component of this milieu, thus "Exposomics" is now being integrated into the study of human disease.<sup>8</sup> Exposomics examines how an individual's lifetime exposures relate to their health and are important in the context of prevention. While a more expansive discussion of environmental and lifestyle precipitators of disease is beyond the scope of this review, this is an area in need of further attention.

The importance of the epigenome and gene-environment interactions in defining expressivity of the genome, and by extension, the impact of genetics on polygenic traits and conditions cannot be overemphasized. Clearly, ENCODE has had a profound impact in advancing precision medicine approaches and the importance of precision prevention strategies to address the challenges posed by complex diseases and health disparities. As such, the one-size-fits-all approach to the practice of medicine is no longer tenable, especially when recognizing that most of what we understand about the intersection of genetics and clinical practice approximates a relatively small number of individuals in the global population.<sup>9,10</sup> This problem is compounded when one considers that predominantly Caucasians are included in data generated from clinical trials, and that limited information is presently available to explain the impact of genetic admixture on human health and disease.<sup>10</sup>

As noted earlier, there is increasing recognition that the study of human health and disease must be carried out at the intersection of molecules, environment, and lifestyle. In the case of molecules, to date most clinical applications have focused on DNA, with less attention given to the biology encoded by RNAs, both coding and non-coding, and the complexity of biological regulation at the level of proteins and metabolites. The latter is particularly important given that the field of metabolomics has not yet been fully integrated with clinical practice, but holds great promise. The totality of this information needs to be integrated in ways that allow for implementation of knowledge-based

**Table 1.** ENCODE: Beyond DNA sequence.<sup>5,6</sup>

Select ENCODE findings	Biological implications
80.4% of the human genome participates in a biochemical RNA and/or chromatin associated event in at least one cell type.	Given that approximately 1% of DNA codes for proteins, more of the genome is utilized than previously expected. DNA previously thought to be 'junk' can now be assigned a biochemical function.
95% of the genome is within 8 kb of a DNA-protein interaction.	DNA-protein interactions are extensive and ubiquitous, which supports the assertion that these interactions play an important biological role
DNA can be categorized into seven chromatin states that have varying functions. Transcriptional activity of promoters is quantitatively correlated to chromatin state and transcription factor binding at promoters.	The genome can now be assigned different functional regions based on its chromatin landscape. These regions can be used to further understand gene expression patterns in health and disease, where DNA sequence provided limited information.
Different cell types have unique patterns of DNase I hypersensitivity and transcription factor binding	DNA-protein interactions are important in determining cell phenotype. Implications for disease, biotechnology, therapeutics.
The majority of the genome is transcribed; even non-coding regions and those thought to be transcriptionally silent	Gene products of 'silent' regions may have more important biological roles than previously anticipated.
Non-coding DNA can regulate the expression of protein-coding genes through long range interactions. Genomic proximity is not a simple predictor for interactions.	Seemingly non-functional DNA may actually exert important regulatory control over the expression of coding regions, regardless of proximity to the given gene.
SNPs are just as likely to be found in non-coding functional regions as in coding regions. Many SNPs associated with disease are in non-coding regions	SNPs in non-coding regions can have profound impacts on gene expression by altering the regulation of other genes

health promotion and prevention strategies that can help address the limitations in healthcare delivery discussed earlier.

## The treatment of COPD and prevention of malignant progression requires a precision-based approach

A widespread disease that requires precision-based approaches is chronic obstructive pulmonary disease (COPD). COPD is now the third leading cause of mortality in the world.<sup>11</sup> In the US, the prevalence of COPD varies considerably by state, with <4% in Hawaii, Colorado, and Utah to >9% in Alabama, Tennessee, Kentucky, and West Virginia.<sup>12</sup> COPD is characterized by poorly reversible airflow obstruction coupled with an abnormal inflammatory response in the lungs to noxious particles and gases. COPD patients have an enhanced or abnormal response to inhaled toxic agents that may result in mucus hypersecretion (chronic bronchitis), tissue destruction (emphysema), and disruption of normal repair and defense mechanisms that culminate in small airway inflammation and fibrosis.<sup>13</sup> These patients are at increased risk of primary lung cancer, as well as poor outcomes after lung cancer diagnosis and treatment.<sup>14</sup> However, no approaches have been described to identify those patients whose genetic makeup may put them at increased risk of malignancy. This is critical gap in current knowledge given that the major cause of COPD, smoking, is a preventable habit and that early identification of those at risk can serve to prevent devastating health outcomes. Of interest in this regard is a recent study that demonstrated an association between carcinogen-DNA adducts in white blood cells and lung cancer risk in the Physicians Health Study.<sup>15</sup> This discovery reinforces the feasibility of precision approaches and advances the study of susceptibility biomarkers.

From a biological perspective, it is important to recognize that a process known as epithelial-to-mesenchymal transition (EMT) is a critical pathogenetic event in COPD.<sup>16</sup> This is particularly significant given that none of the therapies in use today for the management of COPD are directed at the etiology of disease. Instead, the drugs available to manage these patients are directed at symptomatic or palliative relief and prevention of unwanted exacerbations that accelerate disease progression. This problem is magnified when considering that COPD exhibits considerable heterogeneity both in severity of disease, comorbidities, response to treatment, disease progression, and confounders. A summary of the factors contributing to the heterogeneity of COPD is presented in Table 2. A major hurdle to be overcome is the ability to define the critical gene-environment-lifestyle interactions that drive increased risk of disease.

On the same note, there are few predictors that can indicate which COPD patients are most at risk of malignant progression. In 2013, the US Preventive Services Task Force recommended that adults aged 55–80 years with a history of smoking undergo annual screening for lung cancer using low-dose computed tomography (LD-CT).<sup>22</sup> Those to be screened are individuals who have a 30-pack-

**Table 2.** Factors contributing to variable COPD phenotypes.<sup>17–21</sup>

Factor	Heterogeneity
<b>Susceptibility</b>	
Environmental exposures	Occupational agents, viruses, pollution <sup>17</sup>
Smoking history	Past smokers, current smokers, never smokers <sup>17</sup>
Genetics	
COPD alone	<i>FAM13A</i> , <i>HHIP</i> , <i>CYP2A6</i> , etc. <sup>18</sup>
COPD × smoking	<i>CHRNA3/CHRNA5</i> , <i>CYP2A6</i> , <i>DBH</i> , etc. <sup>18</sup>
Sex	Women may be more susceptible to smoking effects <sup>19</sup>
<b>Presentation</b>	
Airflow limitation	Staged by severity of lung function impairment
Symptoms	Self-reported coughing, wheezing
Respiratory components	Chronic bronchitis, asthma, emphysema
Reversibility with treatment	Degree of reversibility
<b>Morbidity and mortality</b>	
Comorbidities	Malnutrition, cardiovascular diseases, anxiety, depression, osteoporosis, obesity, metabolic syndrome, diabetes, sleep disturbance and anemia <sup>20</sup>
Cancer	No cancer, lung cancer, other type of cancer
Ultimate cause of mortality	
COPD-related	Cardiovascular, respiratory (~40% <sup>21</sup> )
Non-COPD related	

year smoking history and currently smoke or have quit smoking within the past 15 years. Screening is to be discontinued once a person has not smoked for 15 years or develops a health problem that substantially limits life expectancy or the ability or willingness to have curative lung cancer surgery. Across three rounds of study, however, it was found that when a positive result was obtained, 96.4% of the LD-CT tests and 94.5% of the chest X-ray exams were false positive, meaning, no lung cancer was present.<sup>23</sup> As such, the challenge with LD-CT precision prevention of lung cancer is the fact that approaches in place are not guided by pathogenesis. To address this challenge, translational and clinical efforts have focused on the identification of molecular targets for diagnostic and therapeutic intervention.<sup>24</sup> These efforts built on work completed nearly 25 years before showing that phenotypic modulation of human and murine somatic cells by chemical carcinogens was associated with reactivation of LINE-1 (Long Interspersed Nuclear Element-1) retro-elements.<sup>25</sup>

## Long interspersed nuclear element-1 as a therapeutic target

The development of precision prevention strategies for COPD and various forms of cancer is being addressed in translational and clinical studies by our research group (Guerra-Ramos Submitted). These efforts have focused on long interspersed nuclear element-1 (LINE-1) as both a target for intervention and a biomarker of disease.

LINE-1 is a ~6 kB retrotransposon that ‘copies and pastes’ itself and other DNAs into different loci throughout the genome via a reverse transcriptase-mediated mechanism. LINE-1 contains two open reading frames downstream of



the regulatory region encoding for two proteins known as open reading frame (ORF1)p and ORF2p. ORF1p is a nucleotide binding protein that is present in ribonucleoprotein particles and shuttles L1 RNA into the nucleus, while ORF2p is a protein with endonuclease and reverse transcriptase activities involved in retrotransposition.<sup>26</sup>

The genome is not static, with ~2% representing coding regions that have been fairly well characterized, and ~45% representing repetitive sequences with variable abilities to undergo transcription and in some instances translation, and to mobilize to different locations via transposition.<sup>27</sup> Of this 45%, nearly 20% is constituted by LINE-1 sequences present as either full length sequences capable of mobilization, or truncated forms unable to complete transposition. Full-length LINE-1 sequences possess the machinery for codification and are able to mobilize when activated via epigenetic mechanisms. In humans, only about 100 active full-length L1 elements remain, with the remainder inactivated via truncation.<sup>25</sup> Full length LINE-1 includes a 5'-untranslated region (UTR) that contains regulatory sequences that recognize proteins such as yin yang 1 (YY1), runt-related transcription factor 3 (RUNX-3), and E2 factor/retinoblastoma tumor suppressor protein (E2F/RB).<sup>28</sup> The latter is particularly significant in light of work completed by our group establishing this complex as one of the master effectors of LINE-1 epigenetic silencing.<sup>29</sup> The silencing of LINE-1 is executed via DNA methylation through a complex process that involves assembly of nucleosome remodeling deacetylase (NuRD) corepressor complexes.<sup>30</sup> These repressive marks can be removed by aberrant endogenous cellular processes and/or exposure to agents that induce DNA damage or perturb the epigenome.<sup>31</sup> Reactivation of LINE-1 can reprogram the genome by causing insertion mutations or deletions that disrupt genome architecture and function.<sup>32–34</sup> Additionally, LINE-1 activation can initiate aberrant gene expression changes that are independent of retrotransposition.<sup>35</sup>

Our group discovered that LINE-1 activation causes airway epithelial cells to acquire a mesenchymal phenotype via EMT, which is mediated by reprogramming of genomes by LINE-1.<sup>36</sup> Further investigation revealed that the tobacco carcinogen benzo[a]pyrene activates LINE-1 through transforming growth factor (TGF)- $\beta$ 1, a master regulator of EMT, via SMAD2/3-mediated canonical signaling and that TGF- $\beta$ 1 inhibitors can block LINE-1 activation.<sup>36</sup> LINE-1 regulates a large number of genes and proteins involved in EMT including N-Cadherin and zinc finger protein SNAIL1 (SNAIL), which in turn, modulate downstream activation/deactivation of important EMT pathways.<sup>36</sup> These findings suggest that LINE-1 and TGF- $\beta$ 1 work in a coordinate fashion to regulate EMT.

This discovery is germane to COPD because EMT drives the pathophysiological changes that lead to lung fibrosis and impaired lung function.<sup>37</sup> In support of this relationship, we recently found that circulating levels of LINE-1 ORF1p in blood are elevated in former smokers diagnosed with COPD based on forced expiratory volume in 1 s (FEV1)/forced vital capacity (FVC) ratios of <70% (Guerra-Ramos Submitted). As such, targeted LINE-1 interventions using small molecule inhibitors may help prevent

the progression of disease, a hypothesis currently being tested in pending clinical trials. The pursuit of “druggable” targets of LINE-1 is work in progress, but the team is optimistic that interventions directed at disrupting uncontrolled LINE-1 functions may represent a successful intervention in modifying disease trajectory and changing clinical outcomes of patients with COPD.

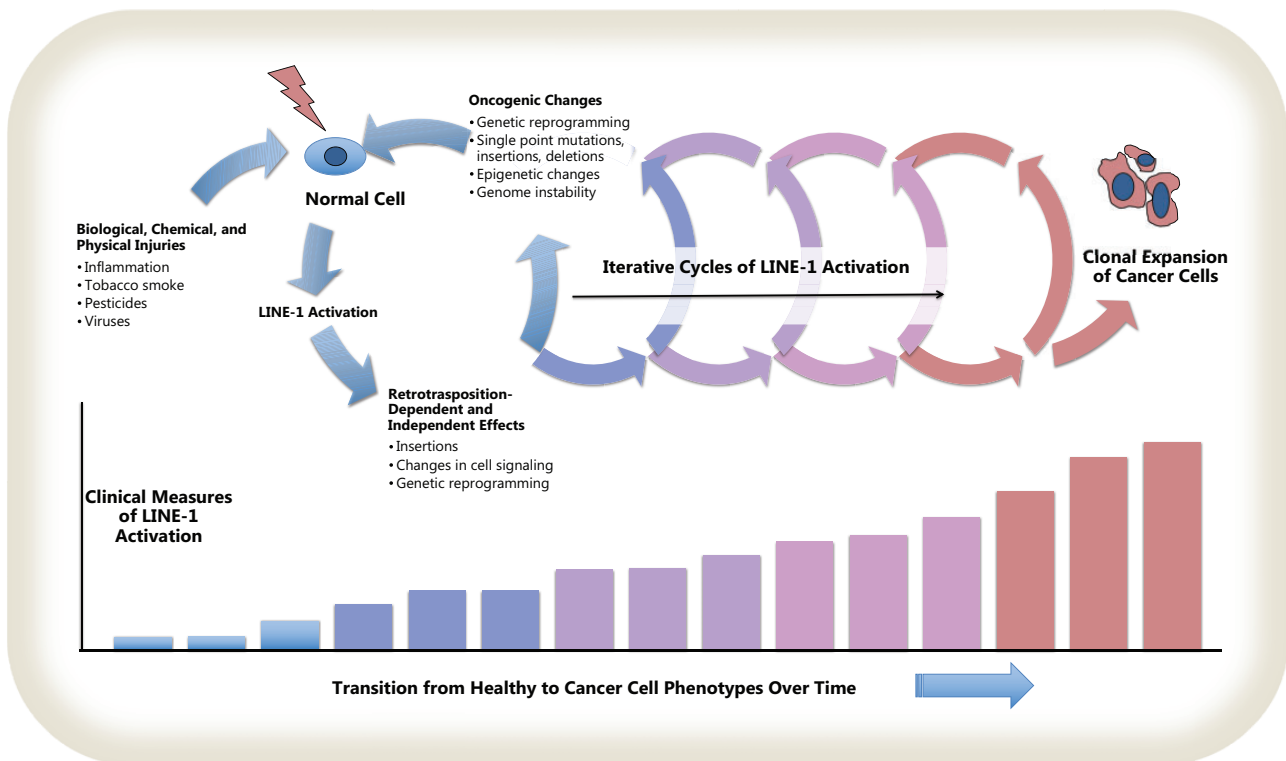
## LINE-1 activation is linked with lung cancer and may serve as a precision biomarker of malignancy

The finding that EMT programming involves the reactivation of LINE-1 is in keeping with the role of these genetic sequences in modulating genomes through insertional mutation or chromatin rearrangements.<sup>28,36</sup> The linkage between EMT programming and malignancy in patients with COPD may thus be related to the modulation of EMT programming by LINE-1.

In addition to insertional mutations, chromatin rearrangements, and the promotion of EMT, additional biological factors contribute to the strong link between LINE-1 activation and cancer development. The proteins encoded by LINE-1 mediate a plethora of ancillary biological functions that modify cell biology, including activation of the mitogen-activated protein kinase (MAPK) and the phosphatidylinositol 3-kinase (PI3K)-AKT pathways,<sup>36</sup> RNA binding, and nucleic acid chaperone activity.<sup>38</sup> Moreover, LINE-1 also alters cellular phenotypes through insertion-independent changes in gene expression. This was discovered when mutant LINE-1 vectors that lack retrotransposition activity were found to modulate the expression of a large number of genetic targets involved in extracellular matrix, inflammation, cellular metabolism, and tumorigenesis.<sup>30,35</sup> Known retrotransposition-independent tumorigenic targets of LINE-1 include Chloride intracellular channel 3 (CLIC3), Nemo-like kinase (NLK), Protein kinase inhibitor alpha (PKIA) Preimplantation protein 4 (PREI4), Cysteine dioxygenase 1 (CDO1), and Ectopic viral integration site 2a (EVI2A).<sup>35</sup>

The strong mechanistic link between LINE-1 activation and cancer development is fully supported by multiple studies demonstrating that LINE-1 activity correlates with increased lung cancer mortality, as shown in studies where LINE-1 hypomethylation correlates with decreased survival.<sup>39–41</sup> It follows suit that the genome of lung cancer cells is one of the most frequently impacted by *de novo* LINE-1 insertions, with >50% of NSCLC having increased LINE-1 ORF1p expression across a panel of different human lung neoplasms.<sup>26</sup> Thus, clinical measures of LINE-1 activation may also serve as an excellent biomarker of lung cancer risk among COPD patients. This rationale is supported by other groups who have taken similar approaches.<sup>42</sup>

In summary, LINE-1 is activated by cellular injury, which promotes EMT and cancer phenotypes through multifaceted mechanisms (Figure 1). Repeated LINE-1 activation cycles over time, as in the case of COPD patients who smoke, can lead to malignant progression. The fact that LINE-1 is both a mediator in this process and exhibits increased activation with severity of disease means that it



**Figure 1.** The role of LINE-1 in the etiology of lung cancer and clinical applications. LINE-1 is activated by various forms of cellular injury, including cigarette smoke and inflammation. The immediate consequences of LINE-1 activation arise from retrotransposition-dependent and independent-processes, that can include as alterations in cellular signaling and LINE-1 insertions. Repeated episodes of LINE-1 activation can have more persistent effects such as epigenetic reprogramming, DNA mutations, genetic reprogramming, etc. that promote oncogenesis. Clinical measures of LINE-1 activation could be used to assess the risk of malignant progression.

can serve as both a therapeutic target and potential biomarker of risk and disease. Ongoing efforts in the laboratory are being directed toward the identification of small molecule inhibitors that can impede the progression of COPD and cancer progression by inhibiting the reactivation of LINE-1. Of interest, are inhibitors of TGF-beta1 known to inhibit LINE-1 activity, EMT, and to modulate disease phenotypes.<sup>36</sup>

## Conclusions

Precision prevention will continue to develop with advances in genome and molecular biology advances. These concepts are being integrated into a conceptual framework depicted in Figure 1. Collectively, the study of LINE-1 and its role in the pathogenesis of COPD and lung cancer may provide valuable diagnostic and therapeutic tools that can be used to identify pre-emptively individuals at risk of COPD and lung cancer. This approach may lead to implementation of preventive measures that spare those impacted by disease from further deterioration and in the process lead to significant cost savings.

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responsibility for appropriate portions of the content; and KSR, EB, MATG, INR have agreed to be accountable for all aspects of the work and ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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## DECLARATION OF CONFLICTING INTERESTS

KS Ramos and EC Bowers have filed a disclosure on the use of LINE-1 as a cancer biomarker in liquid biopsy format. The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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