Editorial

VELUCT, a long non-coding RNA with an important cellular function despite low abundance

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With the completion of the human genome project, it has become obvious that protein-coding genes comprise only 2% of the genome, although the majority of the genome is transcribed into RNA. RNA molecules that lack protein-coding potential are collectively referred to as non-coding (nc) RNAs. In addition to the well-known housekeeping rRNAs, tRNAs and small nuclear RNAs (snRNAs), the most intensively studied subgroup of ncRNAs are the microRNAs (miRNAs), which are well characterized by their size and uniform function. Another distinct class of ncRNAs, long ncRNAs (lncRNAs), are larger than 200 nucleotides. They are markedly heterogeneous in size and cellular function. While the number of annotated lncRNA genes in the human genome outnumbers protein-coding genes (1), studies of their functional roles and detailed mechanisms account for less than 0.1% of all predicted lncRNAs (2).

Lung cancer is the most common cancer diagnosed worldwide. Despite surgical treatment and the development of more effective chemotherapeutics, lung cancer and its metastases are still the leading cause of cancer death in developed countries (3). Efforts to understand differences in patients' responses have led to a focus on gene-expression differences. While initially the role of protein-coding genes and their mutations were studied to understand individual differences in lung cancer formation and to identify novel therapeutic targets (3), the focus in the last decade has shifted to ncRNAs (4,5). In various human cancers—including lung cancer—down-regulation of tumor suppressive miRNAs and up-regulation of oncogenic miRNAs have been described. Lung-cancer-associated miRNAs have the ability to regulate tumorigenesis, survival, angiogenesis, and migration and invasion of tumors (6); moreover, clinical studies have been carried out to correlate dysregulated expression of particular miRNAs with tumor responsiveness to chemotherapies (5). Similarly to microRNAs, lncRNAs were shown to play a fundamental role in cancer-cell growth, proliferation, cell death, invasion and metastasis formation (4); however, the majority of these genes are not yet functionally characterized. Currently, many attempts are focusing on the use of lncRNAs as prognostic markers in cancer patients, as tumor cells can be characterized by their distinct lncRNA profile (7).

In a recent paper, Seiler *et al.* used a siRNA screen to characterize 638 lung-cancer associated lncRNAs (8). Based on the siRNA screen, they selected a yet uncharacterized intergenic lncRNA (ENST00000567151) for further characterization. This lncRNA is overexpressed in primary lung adenocarcinoma and the authors referred to it as viability enhancing in lung cancer transcript (VELUCT). VELUCT expression was low (0.01 copies/cell) in the lung-cancer cell line NCI-H460, and the transcript was predominantly expressed in the chromatin fraction, exhibiting a half-life of 20–30 minutes.

The low abundance of VELUCT raised the question of whether it was functional or just transcriptional

noise. To support the functionality of this transcript, the authors studied the knock-out phenotype. Despite its low abundance, silencing with antisense oligos (ASOs) for VELUCT resulted in a loss of viability of several lung-cancer cell lines. These results demonstrated that even an extremely low abundant lncRNA can have an important function at the cellular level.

Numerous lncRNAs have been shown to affect cell viability; however, the expression of these transcripts is typically much higher than VELUCT. One of the earliest identified lncRNAs, MALAT-1, is overexpressed in metastasizing lung adenocarcinoma and is associated with a high risk of developing metastasis (9). MALAT-1 has since been shown to be upregulated and crucial for cell viability and proliferation in several cancer cell types (10-12). Recently, several other tumor-associated lncRNAs were shown to affect cell viability. The expression of DB327252 in lung tumors is upregulated compared to adjacent normal tissue, exhibiting a magnitude of expression similar to VELUCT, and silencing this transcript inhibited the proliferation of tumor cells (13). In small-cell lung cancer, the taurine up-regulated gene 1 (TUG1) lncRNA was found to be overexpressed, and knockdown of this gene inhibited proliferation, invasion and metastasis formation (14). Ectopic overexpression of the prostate-cancer-specific PCGEM1 lncRNA contributed to cell proliferation (15). In contrast to the sequences mentioned above, some lncRNAs have been shown to be downregulated in cancer tissues. In non-small-cell lung carcinoma, the anti-sense transcript of the tumor suppressor in lung cancer 1 (TSLC1) RP11-713B9.1 was shown to be downregulated, and its functions as tumor suppressor were identified by its ability to decrease cell viability and proliferative signaling when overexpressed (16).

Currently ncRNA biology faces two challenges: the validation of lncRNAs identified by large-scale gene expression studies and the confirmation of their functionality in health and in disease. There is still a debate whether low-abundance lncRNAs are functional or just transcriptional noise that is rapidly removed by cellular quality control mechanisms (17). Although various criteria, including expression levels, splicing variants and sequence conservation, has been proposed to differentiate between functional and non-functional lncRNAs, a consensus has not yet been achieved. Even if a fraction of non-coding transcripts are non-functional and originate from aberrant transcription, thousands might have functions within cells, and an enormous effort is still needed to determine their specific functions. This effort is made more difficult by the low-level expression seen for most lncRNAs (1) and high inter-individual differences in lncRNA expression (18). Seiler *et al.* present one of the first examples of functionality of a low-abundance lncRNA, demonstrating the importance of lncRNAs in small amounts. In addition to individual characterization of lncRNAs, siRNA screening can be a powerful tool to study the functionality of hundreds of lncRNAs simultaneously to find potential new players in various cellular processes, as demonstrated by Seiler *et al.*

lncRNA biology is an emerging topic as evidenced by a 20-fold increase in the number of publications in the last decade (results of a PubMed search with the keyword "lncRNA"). The initial knowledge about a few differentially expressed transcripts has expanded into the functional characterization of over 100 lncRNA species; however, it seems our current knowledge is only the tip of the iceberg. In the future, innovative studies using novel methods— even in the cases where the detection of a transcript first seems dubious—will bring us a deeper understanding of cellular functions and eventually lead to a more complex understanding of disease pathogenesis, possibly providing novel biomarkers and therapeutic targets.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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