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SPOTLIGHT

Long noncoding RNA amplified in lung cancer rewires cancer pathways

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Athie et al. (2020. *J. Cell Biol.* https://doi.org/10.1083/jcb.201908078) identify *ALAL-1*, a lncRNA frequently amplified or overexpressed in lung cancer, as an oncogenic driver, capable of promoting the proliferation and altering the immunogenicity of lung cancer cells.

As vast regions of the cancer genome remain unexplored, it has been proposed that noncoding genes may harbor hidden regulatory elements and serve as therapeutic targets in cancer (1). Long noncoding RNAs (lncRNAs), which are transcripts longer than 200 nucleotides without apparent protein-coding potential, have been a particularly fascinating and promising subject of exploration due to their frequent deregulation in cancer and proposed involvement in cancer pathways (2, 3). However, linking genetic and epigenetic alterations of lncRNAs to functional contributions in tumorigenesis has been challenging for both technical and conceptual reasons. The work by Athie et al. provides a roadmap for how to combine state-of-the-art bioinformatics, genetic, and molecular approaches to begin addressing the question of whether altered lncRNAs are drivers or mere passengers in cancer development.

The presence of recurrent somatic gains or losses of a genomic locus in cancer is one of the strongest indicators for oncogenic or tumor suppressor elements, respectively. By surveying somatic copy number alteration data available from The Cancer Genome Atlas, Athie et al. highlighted 50 lncRNA-containing regions that are frequently amplified or deleted in a tumor-specific manner (4). They hypothesized that the lncRNAs expressed from the altered regions may be functional drivers in cancer and ultimately chose to focus on *ALAL-1* (*Amplified LncRNA Associated with Lung cancer-1*; Fig. 1). *ALAL-1* is amplified in 6–9% of lung adenocarcinoma (LUAD) patients and is frequently overexpressed in lung squamous carcinoma and neck squamous carcinoma, likely due to *ALAL-1* promoter hypomethylation in these tumor types.

To investigate the functional role of *ALAL-1*, the researchers reverted *ALAL-1* amplification in a LUAD cell line with high *ALAL-1* copy number by performing CRISPR-mediated excision of the minimal region of amplification. Strikingly, the reduction of *ALAL-1* copy number and the concomitant down-regulation of *ALAL-1* expression levels led to slower proliferation, diminished colony formation capacity, and increased cell death. These results were replicated using RNAi to knock down *ALAL-1* RNA, suggesting that *ALAL-1* function is mediated through the RNA molecule, rather than through a regulatory DNA element within the amplified region. Conversely, exogenous overexpression of *ALAL-1* in a panel of lung cancer cell lines with or without *ALAL-1* amplification increased their clonogenic and tumorigenic potential. Together, these results showed that the *ALAL-1*-linked region of amplification contains a functional pro-oncogenic element and pointed to a direct sensitivity of cancer cells to *ALAL-1* RNA levels.

Next, Athie et al. characterized the *ALAL-1* transcript. They found that *ALAL-1* is a spliced RNA, comprised of three exons, which is exported to the cytoplasm. They also observed that *ALAL-1* is an NF-kB target, as they identified p65/RelA and NF-kB binding sites and motifs in the genomic region and found that *ALAL-1* expression is sensitive to stimulation or inhibition of this pathway. These findings are not surprising given that *ALAL-1* shares a bi-directional promoter with a known NF-kB target gene, *IKBKB* (5). Indeed, the functional cooperation between the two adjacent transcriptional units is striking and raises interesting questions about convergent functions of neighboring lncRNA and protein-coding genes downstream of a common transcriptional pathway.

Growing data suggest that many lncRNAs directly interact with RNA binding proteins to influence their localization and/or function (6). In an effort to determine the mechanism of *ALAL-1*, Athie et al. identified a direct interaction between *ALAL-1* and SART3, a multifunctional protein, which has been proposed to act as a recycling factor for the spliceosome as well as a substrate-targeting factor for deubiquitinases, such as USP4. The interaction between *ALAL-1* and SART3 was confirmed by RNA pulldown and RNA immunoprecipitation, and the colocalization of the two factors was visualized by confocal microscopy. Furthermore, epistasis experiments revealed that *ALAL-1* and SART3 act together to

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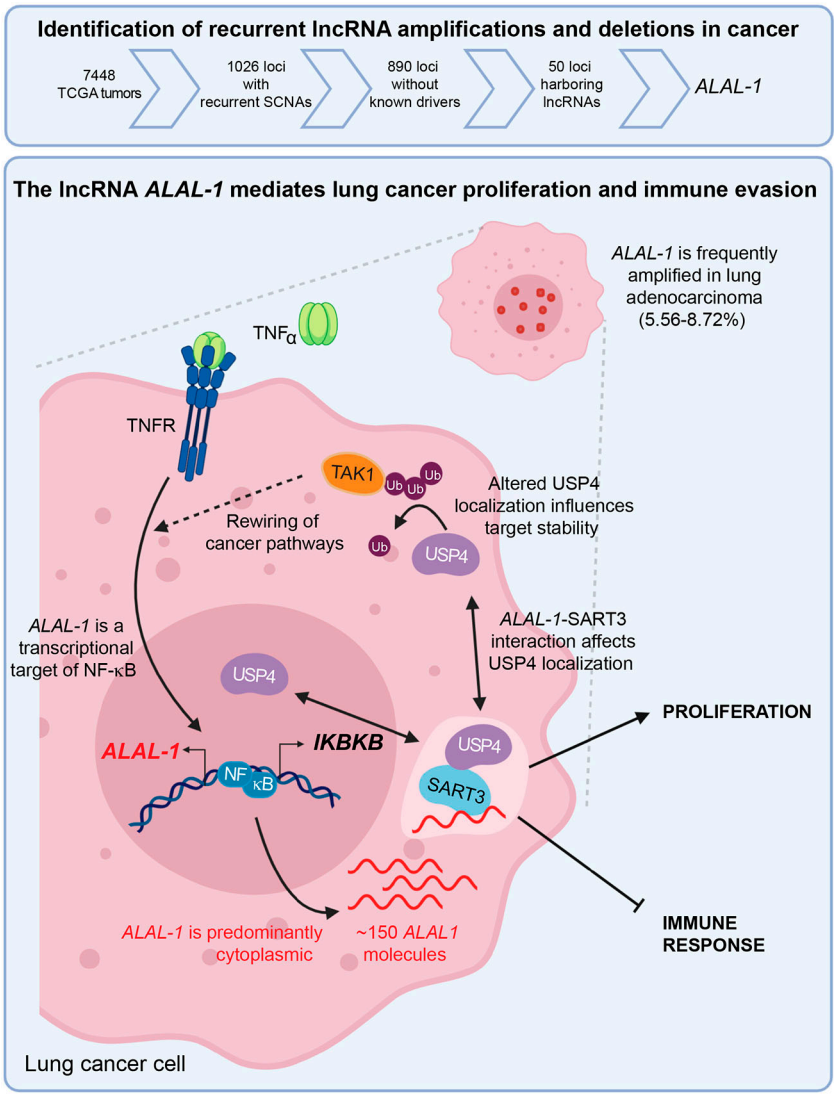

Figure 1. **Amplification of the lncRNA *ALAL-1* promotes proliferation and immune evasion in lung cancer**. Following TNFα treatment, the lncRNA *ALAL-1* is transcriptionally up-regulated and localizes to the cytoplasm, where it interacts with the RNA binding protein SART3. SART3 interacts with the deubiquitinase USP4 in the cytoplasm and regulates USP4 shuttling to the nucleus. The localization and activity of USP4 are affected by the *ALAL-1*-SART3 interaction, which results in changes in the stability of USP4 substrates, including depletion of the cytoplasmic target TAK1 and stabilization of the nuclear target PRP3. Ultimately, amplification of *ALAL-1* favors tumor progression by influencing cellular proliferation and immune response regulation.

promote cellular proliferation as the effects of *ALAL-1* overexpression were reversed upon SART3 knockdown, providing evidence for functional cooperation.

As *ALAL-1* did not appear to modulate the levels or localization of SART3, Athie et al. examined whether the lncRNA influenced factors downstream of SART3. Indeed, they observed that the localization and activity of USP4 was affected by *ALAL-1* knockdown. Specifically, when *ALAL-1* was depleted by RNAi, SART3-mediated nuclear transport of USP4 was impaired. In turn, the altered localization of USP4 affected its ability to regulate the turnover of substrates, including the cytoplasmic TAK1 and the nuclear PRP3. The authors correlated these findings with a global deregulation of several cancer pathways, including TNF, p53, NF-kB, TGF-β1, and IL8, determined by gene ontology analysis of differentially expressed genes in both *ALAL-1* and SART3 knockdown cell lines.

Lastly, given the role of these pathways in immune responses, Athie et al. examined the relationship between *ALAL-1* expression levels and gene signatures of immune infiltration in lung squamous carcinoma samples. The data suggested an inverse correlation between *ALAL-1* amplification and immune signatures, pointing to a potentially immunosuppressive role for *ALAL-1* via modulation of pro-inflammatory pathways.

In summary, Huarte and colleagues identified and characterized *ALAL-1* as a novel pro-oncogenic factor in lung cancer, adding to the growing list of functional lncRNAs in cancer. The potential involvement of *ALAL-1* across many different cancer pathways merits further investigation. Indeed, it will be crucial to determine the impact of targeting *ALAL-1* on both tumor growth and tumor immunogenicity. It will also be important to study and possibly differentiate the roles of *ALAL-1* in normal cells versus the novel roles that *ALAL-1* may acquire in cancer cells in the context of amplification and overexpression.

This work highlights the pressing need to develop methods to investigate the functional elements of lncRNAs, such as *ALAL-1*, in order to narrow down targetable elements in a therapeutic setting. Unfortunately, this problem has traditionally proven challenging. With few exceptions, there is an almost complete lack of knowledge about how specific lncRNA sequences or structures affect their functions, such as localization or interactions with binding partners. While it is possible that *ALAL-1* may function through its putative SART3-binding sites, as proposed by Athie et al., it is also conceivable that it may act in a largely sequence-independent manner, perhaps by promoting the aggregation of SART3 (7). Alternatively, the data do not exclude the possibility that cytoplasmic *ALAL-1* may have peptide-encoding capacity, which contributes to its functions (8). Studying *ALAL-1* is further confounded by the complexity of the *ALAL-1* locus, which harbors multiple overlapping transcripts and shares regulatory elements with the neighboring *IKBKB*, a key mediator of the pathways that *ALAL-1* also appears to modulate. Understanding the molecular basis for the function of *ALAL-1* and other lncRNAs will take painstaking efforts, such as the generation of precise genetic models as well as the development of myriad deletion and point mutants, which possess or lack activity. These efforts are key to understanding

the functional roles of lncRNAs in normal and disease states, such as cancer, and to harnessing their potential for therapeutic purposes.

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