Letter to the Editor

Interactome analysis reveals MALAT1 binding with DBC1—author's reply

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By now, thousands of long non-coding RNAs (lncRNAs) have been reported to be differentially expressed in different cell types, and involved in regulations of diseases through different mechanisms (1-3). LncRNAs modulate cell functions mainly by interactions with bio-macromolecules, including proteins (4).

There are two ways to explore interaction of lncRNA and protein, protein-centric and RNA-centric. Protein-centric method is widely used by combination of RNA-sequencing with RNA immunoprecipitation or crosslinking-immunoprecipitation. Obviously, this method could not provide a comprehensive profile of lncRNA interactome and require prior knowledge of proteins. RNA-centric method is usually needed to explore unknown functions of lncRNAs (5). In our study, a high-throughput strategy was employed to characterize the interactome of metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) by combining RNA pulldown, quantitative proteomics, bioinformatics, and experimental validation. We find that MALAT1 competes with sirtuin1 (SIRT1) to bind with DBC1, sequentially decreases the deacetylation of p53 and inhibits a spectrum of p53 downstream biological function. Therefore, we put forward a novel mechanism by which MALAT1 decreases p53 activity through lncRNA-protein interaction (6).

The whole length of MALAT1 is over 8,000 nt, therefore it is very difficult to construct the complete transcript via *in vitro* transcription. We used the 6,918–8,441 nt region of MALAT1 to perform the pulldown assay. This fragment contains the homologous sequence of MALAT1 among different species (7). This conserved domain plays a key role for the functions of MALAT1, and it is widely used for investigating the biological functions of MALAT1 (8). Although we initially identified the interaction between MALAT1 and DBC1 using a fragment of MALAT1, we did verify the results using *in vivo* assays, such as RNA immunoprecipitation. It's unclear whether the 3' region of MALAT1 contains a nuclear localization signal, and whether the binding to DBC1 affects MALAT1 nuclear retention requires further investigation. To study the localization of MALAT1 in HepG2 cell, fluorescence *in situ* hybridization (FISH) was performed. Similar to other types of cell also in HepG2 cells (*Figure 1*). Although several studies implicate the role of MALAT1 as competing endogenous (ceRNA) that modulates the functions of miRNAs, so far there's no solid evidence showing the localization of MALAT1 in the cytoplasm.

As the reviewer commented, our observations were made in HepG2 cells, however it would be interesting to conduct the *in vivo* experiments and test the findings in other cell

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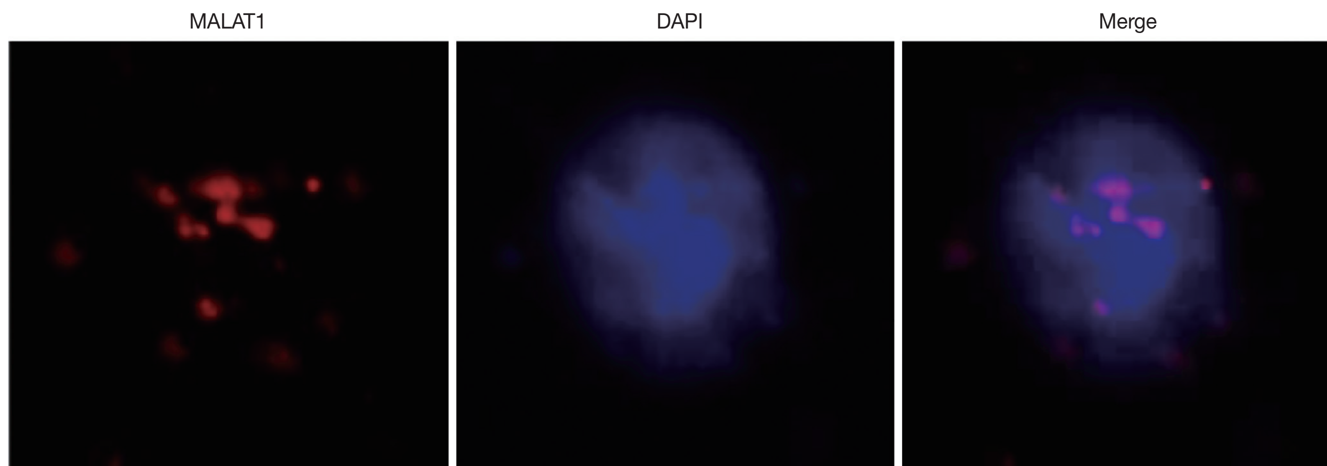

Figure 1 Localization of MALAT1 in HepG2 cells. To detect MALAT1 RNA, cells were rinsed briefly in PBS and then fixed in 4% formaldehyde for 10 min at RT. Further, the cells were permeabilized in PBS containing 0.2% Triton X-100 for 10 min at RT; washed with PBS 3×10 min and rinsed once in 2× SSC prior to hybridization. 6,918–8,441 fragment of MALAT1 antisense was transcribed in vitro, labeled with biotin and used as probes. Hybridization was carried out in a moist chamber at 42 ℃ for 2 hours. After incubated with biotin-labeled Alexa Fluor 546-conjugated secondary antibody, fluorescence mages were acquired using confocal laser scanning microscopy (Olympus FV1000). MALAT1, red color. MALAT1, metastasis-associated lung adenocarcinoma transcript 1.

types. In summary, it is worthy of further investigation to clarifying the biological roles of MALAT1 based on our finding of the MALAT1 protein interactome.

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Footnote

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

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