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COMMENTARY

MALAT1 long non-coding RNA and breast cancer

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ABSTRACT

Non-coding RNAs are becoming major players in disease pathogenesis such as cancer. *Metastasis Associated Lung Adenocarcinoma Transcript 1 (MALAT1)* is a nuclear enriched long non-coding RNA that is generally overexpressed in patient tumors and metastases. Overexpression of *MALAT1* has been shown to be positively correlated with tumor progression and metastasis in a large number of tumor types including breast tumors. Surprisingly, a recent report by Kim et al shows a metastasis suppressive role for *Malat1*. Here, we discuss these results in the context of a large body of published literature that support a pro-tumorigenic role for *MALAT1* in order to gain potential insights into the basis of these observed differences.

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Large-scale genome-wide studies have indicated that thousands of RNAs lacking protein-coding capacity are transcribed from mammalian genomes [1]. A sub-set of these non-coding RNAs are greater than 200 nucleotides in length and are referred to as long non-coding RNAs (lncRNAs). Many lncRNAs have been demonstrated to play a critical role in one or several hallmarks of cancer, including uncontrolled proliferation, evasion of cell death, angiogenesis, immune suppression and/or metastasis [2,3]. Several lncRNAs such as *HOTAIR, H19, PVT1, SChLAP1, LUNAR, NEAT1*, and *MALAT1*, have been recurrently associated with different types of cancers (reviewed in [2,3]). The aberrant expression of these transcripts has been associated with tumorigenesis, metastasis, tumor progression, therapy response and overall survival (reviewed in [2,3]).

MALAT1 (*Metastasis Associated Lung Adenocarcinoma Transcript 1*) is a highly conserved nuclear localized lncRNA transcribed from human chromosome 11q13, it is expressed in most normal human and mouse tissues [4,5], and is prone to copy number changes in several cancer types [6,7]. *MALAT1* was initially identified as a lncRNA whose expression is elevated in primary human non-small cell lung tumors that had a higher propensity to metastasize [5]. Subsequently, *MALAT1* has been shown to be highly expressed in numerous other human cancers including, but not limited to, lung, breast, ovarian, prostate, cervical, endometrial, gastric, pancreatic, sarcoma, colorectal, bladder, brain, hepatocellular carcinoma, esophageal squamous cell carcinoma, renal cell carcinoma, multiple myeloma, and lymphoma (reviewed in [8]).

Interestingly, *Malat1* knockout mice developed by three independent research groups demonstrated that *Malat1* is dispensable for normal development, growth, and viability of the organism [4,9,10], possibly due to redundancy. However, studies using antisense oligonucleotide (ASO) knockdown and/or genetic knockout (KO) of *MALAT1* in lung and breast cancer cell lines and animal models demonstrated impaired cell migration, tumor progression, and significantly reduced metastasis [11,12]. Consistent with these findings in mice, an elevated level of *MALAT1* in breast cancer has been shown to correlate with increased tumor size and stage, as well as poor prognosis in human patients [8,13–16]. However, a recent study by Kim et al., demonstrated an opposite role for *MALAT1* as a suppressor of cell migration and metastasis [17]. An earlier study by Kwok et al. had also shown a non-canonical tumor suppressive role for *MALAT1* in breast cancer [18]. In addition, a study by Eastlack et al showed that *Malat1* can act as a tumor suppressor gene in breast cancers expressing sufficient levels of Nischarin, which itself is a tumor suppressor [19]. While these studies are counter to a very large body of published literature on various aspects of *MALAT1* biology in breast and other cancer types, these findings should be examined carefully as they may reveal new insights into the intricacies of the role of *MALAT1* in various cancer contexts.

MALAT1 has been is shown to promote tumor progression and metastasis in various cancer types (reviewed in [8]) . For example, *MALAT1* knockdown using ASOs in a lung cancer xenograft model and *MALAT1* KO using zinc finger nucleases in a lung cancer cell line resulted in significantly reduced homing and metastasis in these models [12]. Also, gain- and-loss-of-function studies revealed that *MALAT1* promotes the progression of hepatocellular carcinoma by modulating the SRSF1-mediated oncogenic splicing program [20]. Further, Arun et al. [11] demonstrated that genetic loss of *Malat1* in the *MMTV-PyMT* mouse model of breast cancer resulted in significant differentiation of primary tumors and nearly 80% reduction in the incidence of lung metastases. A similar effect was recapitulated using ASO-mediated knockdown of *Malat1*

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in the same model system using two different ASOs [11]. In addition, *Malat1* loss in organoids derived from MMTV-PyMT or Her2/neu-amplified mammary tumors exhibited reduced cell migration, differentiated acinar-like morphology, and increased cell adhesion similar to the *Malat1* knockdown/knockout tumors whereas normal mammary organoids treated with the same ASOs exhibited no altered phenotype [11]. In contrast, Kim et al. [17] found an opposite phenotype upon loss of *Malat1* in the same mouse background MMTV-PyMT: they observed an increase in metastasis and cell migration and this was rescued by transgenic over-expression of full length *Malat1*. Further, they show that CRISPR deletion clones of *MALAT1* in bioluminescent human MDA-MB-231 poorly-differentiated triple negative breast cancer cells resulted in increased metastatic incidence which can again be rescued by full-length mouse *Malat1*. Loss of *MALAT1* specifically seems to affect metastasis in these instances while there is no effect of *MALAT1* loss on primary tumor growth or histology and in overall survival of the mice.

One major discrepancy between the previously published works and the present work by Kim et al [17] seems to emerge from the use of two different approaches in developing the *Malat1* KO mice and CRISPR deletion clones. The study by Arun et al. [11] used KO mice that lacked a 1.3 kb region upstream of the *Malat1* transcription start site (TSS), the TSS, and a 1.7 kb region downstream of the TSS [4]. Kim et al. [17] used KO mice that retained the *Malat1* promoter including the TSS and contained a lacZ construct with a polyA tail inserted 69 nt downstream of the *Malat1* TSS [9]. While both original KO models did not have any apparent phenotypic abnormalities, it is important to note that the *Malat1* KO used by Arun et al. [11] lacked transcription from the *Malat1* locus, whereas the *Malat1* KO generated by Nakagawa et al. [9], and used in the Kim et al. [17] study, retained *Malat1* transcription and synthesis of a ~ 69 nt *Malat1* 5′ fragment. In addition, CRISPR derived clones retained *MALAT1* transcription including synthesis of an ~800 nt 5′ transcript. It needs to be determined whether these differences contributed to the opposing phenotypic outcomes. Kim et al. [17] attribute the observed difference to the effect of *Malat1* promoter loss on several adjacent genes (*Neat1, Scyl1, Cdc42ep2, Ltbp3* etc.) which were upregulated 1.5–2.3 fold in the KO mice generated by Zhang et al. [4]. However, this upregulation was only noted in the brain tissue in these mice.

Translocation of the 5′ region of *MALAT1* to *GLI1* was shown to drive over-expression of the fusion-gene in an aggressive form of gastroblastoma resulting in activation of the Sonic Hedgehog pathway [21]. While *GLI1* is a known oncogene, the role of the *MALAT1* fusion in the pathogenesis of gastroblastoma is intriguing and suggests a critical role for *MALAT1* in disease progression. Furthermore, overexpression of a *Malat1* fragment (2446nt- 2738nt) was found to be sufficient to transform mouse primary embryonic fibroblasts resulting in increased colony formation in soft agar assays [22]. Additionally, a study by Gao et al. demonstrated a *Malat1* fragment (1040nt- 2137nt) derived from metastatic 4T1 cells induces metastasis in the isogenic non-metastatic 4T07 murine breast cancer cell line suggesting a gain of function for this *Malat1* fragment in promoting metastasis [23]. Surprisingly, Kim et al. [17] report that overexpression of *Malat1* in metastatic 4T1 cells reduces lung metastasis.

Multiple scenarios have been proposed for *MALAT1* function in different cellular contexts, including but not limited to epigenetic regulation [12,24] regulation of alternative pre-mRNA splicing [25], transcription regulation during serum response [24], associating with the TSS and the 3′-end of actively transcribing genes [26], sponging miRNAs (reviewed in [8]), or acting as a molecular scaffold [11]. *Malat1* has been shown to bind to a number of nuclear proteins including core spliceosomal proteins, polycomb proteins, and components of the transcription machinery (reviewed in [8]). Kim et al. [17] identified Tead1 as a *Malat1* binding protein and demonstrated that sequestration of Tead1 by *Malat1* prevents its interaction with Yap, a known oncogene, thereby preventing the transcription of pro-metastatic gene signatures. Given the abundance of *Malat1*, sequestration of Tead1 by *Malat1* seems to be a feasible mechanism, yet the cancer-specific role of this interaction is unclear. Given the importance of the Tead family and Yap in development and the lack of any developmental defect upon *Malat1* KO confounds this proposed model and leaves open the possibility of multiple alternative functions and/or redundancy in the function of *Malat1*. Kim et al. [17] further demonstrated that knockdown of *MALAT1* allows TEAD1 to bind to YAP thereby promoting transcription of pro-metastatic genes such as *VEGF-A and ITGB*. However, earlier reports demonstrated a positive role for *MALAT1* in *VEGF-A* regulation. Pruszko et al. [27] show that the oncogenic splicing factor SRSF1 cooperates with *MALAT1* to recruit mutant p53 and ID4 proteins, favoring its chromatin association and thus inducing the expression of various *VEGF* isoforms, indicating a role of *MALAT1* in the promotion of angiogenesis [27]. Further, it has been shown previously by multiple groups that hypoxia inducible transcription factor HIF1b regulates *MALAT1* which is upregulated during hypoxic stress [28,29]. Zhang et al. [28] have shown that *Malat1* regulates cell-autonomous angiogenesis through direct regulation of *VEGFR2* in genetic as well as cell line models. Therefore, it still remains an open question as to whether *MALAT1* positively or negatively regulates pro-metastatic VEGF isoforms.

Although Kim et al. [17] report that *MALAT1* is down-regulated in TCGA datasets of human breast tumors compared to normal tissues, and that *MALAT1* expression in metastasis is lower than in primary tumors, these meta-analyses do not agree with numerous prior studies [11,13,15,16,30–32]. For example, Jadaliha et al. [13] reported that despite overall low expression of *MALAT1* in TNBC tumors, disease free survival was significantly worse in tumors from TNBC patients diagnosed with lymph node negative breast cancer that displayed the top quartile of subtype-specific *MALAT1* expression [13]. Furthermore, an earlier study evaluating breast cancer patient samples, showed that *MALAT1* expression is higher in breast tumors as compared to adjacent normal tissues [30]. More recently, Arun et al. [11] demonstrated that *MALAT1* level is higher in primary breast tumors than in stroma using RNA FISH of human patient tissue sections, its level increases with tumor stage (Arun et al., unpublished data), and it is at least 2–3 times higher in lung and brain metastases when compared to matched primary luminal breast tumor sections.

Interestingly, Arun et al. [11] identified *MALAT1*-high and *MALAT1*-low cells in primary tumor sections and therefore depending on the ratio of such cells the overall level of *MALAT1* reported represents an average when analyzing total tumor RNA (i.e. TCGA or other databases). Another study by Tian et al showed that patients whose primary breast tumors exhibit a high expression of *MALAT1* had a shorter overall survival [31]. More recently, Wang et al. [16] reported that high levels of *MALAT1* are associated with breast cancer relapse in ER+ tumors. In addition, meta-analysis performed on unstratified TCGA breast cancer samples, GEO datasets, as well as independently acquired datasets revealed no statistically significant association between *MALAT1* level and survival [16]. However, when survival analysis was limited to only ER+ individuals, *MALAT1* low expression was found to be significantly associated with relapse-free survival, whereas those ER+ patients with high *MALAT1* levels had a 44% higher risk (95% confidence interval) for relapse compared to patients with low *MALAT1* level [16]. Additionaly, Miao et al [32] have demonstrated using human patient samples that *MALAT1* expression was significantly up-regulated in breast tumors. Furthermore, elevated *MALAT1* expression in breast cancer tissue was significantly associated with lymph metastasis and adverse 5-year Disease Free Survival [32]. Thus, stratifying patient subpopulations and considering the origin of the RNA being analyzed – whether from a bulk tumor or from single cells – is an important consideration when analyzing these types of sample data.

The biggest open question that we are left with is why an RNA implicated in a pro-tumorigenic role in numerous solid tumors, including breast tumors, and some lymphoid tumors would also play an opposing role in breast cancer. Given that the process of metastasis is relatively similar for all cancer types with regard to molecular changes and gene expression, *MALAT1* as a suppressor of metastasis in breast cancer is a rather surprising observation.

While the Kim et al. [17] findings are contrary to most previous findings supporting an oncogenic role of *MALAT1*, in regard to its role in proliferation, migration, metastasis, and poor prognosis (reviewed in [8]), they did perform experiments using more than one model system and importantly rescue experiments appear to validate their findings. As lncRNAs such as *MALAT1* are poised to become important therapeutic targets for breast and other cancer types, it is extremely important to fully investigate and understand the reason(s) for the observed differences of the role of *MALAT1* in tumorigenesis.

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