Editorial

The new role of lncRNA MALAT1 as a tumor suppressor

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Introduction

The research about long non-coding RNAs (lncRNAs) and their effect on the hallmarks of cancer is gaining more and more importance in recent years. lncRNA and messenger RNAs (mRNAs) are distinguished by their ability to translate the transcript into proteins (1). Instead, these non-coding and over 200 nucleotides long ribonucleic acids play significant roles in regulation of transcription, translation, chromatin remodeling and several intracellular pathways (2). Dysregulated expression of lncRNAs in cancer cells shows a direct correlation to the extent of growth, invasion and metastasis in many different cancer types and thus influences the prognosis and therapy response (1).

MALAT1 (metastasis-associated lung adenocarcinoma transcript 1) is one of the most conserved lncRNAs that was first described in non-small cell lung cancer (NSCLC). MALAT1, also known as nuclear-enriched transcript 2 (NEAT2), is about 8 kb long and located at 11q13. MALAT1 has been extensively studied and was described as a possible biomarker due to the effects on the formation of metastasis (2,3). In addition, previous studies have shown that MALAT1 is a metastasis promoting lncRNA, that leads to tumor invasion and growth. In context and completely opposite to what was known, new insights by Kim *et al.* demonstrate that MALAT1 suppresses the induction of metastasis and acts as a tumor suppressor rather than a promoter (3,4). In this editorial we will discuss the general function of MALAT1 in breast cancer (BC) and the new challenging results in the context of recent publications.

The role of *MALAT1* in angiogenesis

In a recent published study, Huang *et al.* have shown that the expression of MALAT1 is increased in BC cells in contrast to healthy breast tissue. MALAT1 was knocked down in MCF-7 cells by using short hairpin RNA (shRNA) to investigate the effects on carcinogenesis. The downregulation of MALAT1 results in lower proliferation and migration of MCF-7 cells *in vitro* (5). The expression of vascular endothelial growth factor (VEGF) was significantly lower due to the knockout of MALAT1. These results stand in contrast to the findings of Kim and colleagues who showed that VEGFA is up-regulated in their models system of MALAT1 knock-out (3). Additionally, the expression levels of miR-145, a microRNA known as a possible tumor suppressor in BC, were determined. miR-145 had a significantly lower expression in BC than in non-cancerous tissues and thus a negative correlation between miR-145 and MALAT1 expression in BC was found. There are a direct interaction and specific binding sites between these RNAs, so that the author hypothesized that cancer cells with high expression of MALAT1 causes a downregulation of miR-145 and thus a lower inhibition of angiogenesis (5).

In colorectal cancer (CRC), angiogenesis and epithelial-mesenchymal transition (EMT) are regulated by YAP1-MALAT1-miR126-5p axis. Knockdown of Yes-associated protein 1 (YAP1) leads to a downregulation of MALAT1 and inhibition of migration and invasion in HCT116 cells. Luciferase activity assays showed that a suppression of YAP1, TCF-4 or β-catenin results in less MALAT1

promotor activity (6). MALAT1 interacts with miR-126-5p at two binding sites and sponges this tumor suppressor. In further consequence miR-126-5p is not able to bind on several mRNAs like VEGFA, SLUG or TWIST. Increased expression of MALAT1 leads to higher protein levels of VEGFA, SLUG and TWIST and enhances the ability to form new vessels and forces the EMT (6).

Due to the important role of MALAT1 in regulation of angiogenesis, it seems to be a possible therapeutic target to reduce the growth rate of cancer cells.

MALAT1 promotes cell proliferation in BC

Zhang *et al.* described an overexpression of MALAT1 in BC tissue, which has a correlation to the progression of BC (7). Besides the elevated MALAT1 levels in BC cells, MALAT1 was also significantly increased in the serum of cancer patients compared with the serum of healthy volunteers. The influence on proliferation was examined in MDA-MB-231 and ZR-75-1 cells by using CCK8 proliferation assay. Transfection with MALAT1-siRNA leads to a suppression of cell proliferation in both cell lines and to a lower tumor weight in average (7). In addition, the expression level of MALAT1 was measured in exosomes, due to its important role in intercellular communication. MDA-MB-231 and ZR-75-1 cells treated with exosomes from MCF-10A cells result in high MALAT1 levels in recipient cells. Treatment with isolated exosomes of MCF-10A cell line led to the same result. This suggests that MALAT1 can be transferred to other cells via exosomes and thus altering proliferation (7).

Xiping *et al.* investigated the effects of MALAT1 on the proliferation of Her-2 positive and triple-negative breast cancer (TNBC). There was no significant coherence between MALAT1 expression levels and overall survival. However, a positive correlation between MALAT1 overexpression and the staging of lymph nodes was found, so that MALAT1 can be considered as a prognostic factor for metastasis (8). The expression of MALAT1 was significantly higher in high-grade metastasis than in BC with poor metastasis. The detection of X-box binding protein 1 (XBP1) and hypoxia-inducible factor 1-alpha (HIF-1α) by quantitative real-time polymerase chain reaction (qRT-PCR) have shown that these two proteins are increased in BC with high MALAT1 levels. *In vivo* the development of BC metastasis is progressing faster under anaerobic conditions. MDA-MB-231 and MDA-MB-435 cells were cultured under anaerobic conditions to determine the expression levels of XBP1 and HIF-1α as well as the proliferation rate. CCK8 analysis revealed that only the proliferation of MDA-MB-231 was increased. In experiments without oxygen treatment, there was a higher expression of MALAT1, XBP1 and HIF-1α in both cell lines but significantly more elevated at MDA-MB-231 (8). MALAT1 was knocked down with three specific siRNA in MDA-MB-231 cells under anaerobic conditions as well as in MDA-MB-435 cells under normoxic conditions and thus led to less proliferation and invasion of both BC cell lines. Based on the results that MALAT1 knockdown do not affect the expression level of XBP1, MDA-MB-231 cells were treated with XBP1-siRNA to study the influence of XBP1 on MALAT1 and HIF-1α. Xiping *et al.* came to the conclusion that MALAT1 promotes the proliferation of BC through a regulation by XBP1 and HIF-1α (8). Further studies have shown that knockdown of Her-2 receptor also decrease the expression of MALAT1 in MDA-MB-435 cell under normoxic conditions. There is the possibility that MALAT1 influences the immune escape of BC due to downregulation of checkpoint genes like *MYC* or *CD47* (8).

A study performed by Zuo *et al.* have shown that MALAT1 was increased in all cultured TNBC cell lines (MDA-MB-231, MDA-MB-453, MCF-7, TB-549, BT-474) and thus MALAT1 also enhances the proliferation and invasion of TNBC (9). In contrast to studies by Xiping *et al.*, they found a significant negative correlation between high MALAT1 levels and overall survival of patients with TNBC (8). Knockdown of MALAT1 in MDA-MB-453 cells with MALAT-siRNA led to less migration, invasion and proliferation *in vitro* because of cell cycle arrest in G1/G0 phase. In addition, MALAT1 knockdown increased the expression of miR-129-5p, which can bind directly to MALAT1, but on the other hand overexpression of miR-129-5p cannot influence the expression of MALAT1. miR-129-5p inhibitors reduce the cell proliferation of MDA-MB-231 cells and this indicates another way in which MALAT1 regulates proliferation by sponging miRNAs (9).

MALAT1 as a potential biomarker

Studies have shown that more than 1,300 lncRNAs differ in their expression levels in BC tissue compared with healthy breast tissue and thus play a crucial role in the estimation of progression of cancer (10). MALAT1 is considered as a biomarker that worsens the prognosis due to its effects on proliferation, invasion and metastasis. MALAT1 expression is upregulated in most estrogen receptor (ER) positive

BC. Although no connection between high MALAT1 expression and tumor grade was found, but there was a correlation between high expression of MALAT1 and ER upregulation (11). Huang *et al.* revealed that the recurrence-free survival (RFS) of patients with ER-negative BC is higher, when the MALAT1 expression is low. However, no significant difference in RFS was found in ER-positive group. High MALAT1 expression led to bad response of tamoxifen in ER-positive BC and thus MALAT1 can be a possible marker for the sensitivity of hormone therapy (11).

Wang *et al.* also described an association between MALAT1 expression and clinical outcome of BC patients. Patients with ER-positive as well as progesterone receptor (PR)-positive BC had a higher expression of MALAT1 and again there was a positive correlation between risk of relapse and upregulation of MALAT1. By incorporating clinical variables into analysis Wang *et al.* revealed that high MALAT1 levels increase the mortality in a dose-dependent manner. This connection was particularly visible at low grade tumors and BC with positive hormone receptor status (12).

MALAT1 and BC metastasis

In addition to the ability to influence proliferation and angiogenesis, MALAT1 is a regulating factor for several processes of metastatic genesis, such as EMT and the induction of matrix metalloproteinases and mesenchymal markers (13). A study performed by Latorre *et al.* described that the complex of RNA binding protein HuR and lncRNA MALAT1 regulates the expression of cancer stem cell marker CD133 (14). In mammospheres MALAT1 is able to bind on regulatory regions of the *CD133* gene and HuR is important to stabilize this interaction. A downregulation of MALAT1 or HuR led to higher expression of CD133. However, MALAT1 does not change the expression level of HuR. Compared with the epithelial BC cell line MCF-7, MDA-MB-231 cells have shown a downregulation of MALAT1 and vice versa a higher expression of CD133 due to the lower repression of HuR/MALAT1 complex. MALAT1 upregulation inhibits the expression of CD133 and subsequently leads to suppression of EMT due to the regulation of prometastatic genes (14).

Other results have also shown that the downregulation of MALAT1 have reduced BC metastasis. MALAT1 knockout mice were crossed with MMTV-PyMT mice to investigate the differences in metastasis. Due to the elimination of the *MALAT1* gene no micrometastases were found and the number of macrometastases in the lungs were reduced (15). In addition, knockdown of MALAT1 with antisense oligonucleotides (ASOs) led generally to lower growth and to changes in tumor morphogenesis like increased cell adhesion and reduced migration. Integrins such as Itga2b and Itga5b3 are downregulated and many other proteins had different expression levels at tumors with low MALAT1 expression based on the hypothesis that MALAT1 can interact directly with regulatory regions of those genes. The usage of ASOs in the therapeutic field could be a possibility to reduce metastasis of BC by downregulating MALAT1 (15).

In their recent paper, challenging and controversial results have been published by Kim *et al.* (3). In contrast to previous studies, the authors suggest that MALAT1 is a tumor suppressor of BC metastases. The authors argued that in previous studies and models the knock-out of *MALAT1* gene leads to upregulation of adjacent genes which may have influenced previously findings substantially. Kim *et al.* selected a new approach where a terminator region was inserted after the transcriptional start site of MALAT1 to abolish the MALAT1 RNA expression (3). In this case, the expression levels of the neighboring genes stay the same and only MALAT1 was decreased. MMTV-PyMT mice with MALAT1 knockout had an increased number of circulating tumor cells (CTC) compared with control group. Knockout mice with rescued transgenic expression of MALAT1 showed no differences to wild type mice in terms of tumor growth and metastasis. This rescue experiment confirms the suppressive function of MALAT1 in an new and very promising manner (3). Chromatin isolation by RNA purification and western blot analysis have shown that all members of TEAD protein family can interact with MALAT1. TEAD1 and its coactivators YAP and TAZ are responsible for the tumor progression by activating tumor and metastasis promoting genes. MALAT1 binds to the same domain of TEAD1 as YAP and thus blocks the YAP-TEAD1 interaction. In further consequence metastasis promoting proteins like vascular endothelial growth factor (VEGFA) and integrin β4 (ITGB4) are downregulated due to interaction of MALAT1 with TEAD1 (3). Overall, the study by Kim *et al.* destroys a paradigm and is in strong contrast to almost all other published studies. The authors used a multiple line of experiments and model systems to underline their hypothesis, thus convincingly showing and establishing MALAT1 as a BC tumor suppressive factor. According to the authors, almost all other studies on MALAT1 used wrong or insufficient model systems, MALAT1 targeting strategies and generated wrong results and concluded hypothesis. Therefore, the paper from Kim

et al. is of outstanding novelty and reverses a dogma in long non-coding RNA biology.

Conclusions

Many studies in recent years have demonstrated without doubt the importance of lncRNAs in carcinogenesis. There are a number of ways to treat cancer patients in a new way, or at least to be able to estimate their prognosis. The crucial role of MALAT1 in regulation of several cancer promoting pathways has been proposed by a series of studies and its use as a biomarker in future has been postulated. The current study of Kim *et al.* shows contrary results to many published articles. The properties of MALAT1 should now be considered from this new perspective to understand the full potential of BC therapy by targeting MALAT1.

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Footnote

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

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