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Canonical and non-canonical functions of METTL3 in breast cancer

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Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för avläggande av filosofie doktorsexamen framläggs till offentligt försvar i Major Groove Department of Molecular Biology, University hospital area, building 6L.
Tiesdag den 13 December, kl. 09:00.

Avhandlingen kommer att försvaras på engelska.

Fakultetsopponent: Associate Professor, Jean-Yves Roignant, Centre for Integrative Genomics, Université de Lausanne, Switzerland.

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Abstract
Gene expression is spatially and temporally regulated at multiple levels. N^6-methyladenosine (m^6A) is the most prevalent internal modification in messenger RNA (mRNA) and long noncoding RNA (lncRNAs). m^6A plays important roles in multiple cellular processes including stem cell pluripotency, adipogenesis, spermatogenesis, neurogenesis, circadian rhythm and development by modulating RNA splicing, export, stability, degradation and translation. Although aberrant m^6A methylation has been reported in various types of cancer, the underlying molecular functions of METTL3, the solely catalytic subunit of the m^6A-methylase complex, has yet to be defined.

m^6A has been recently identified in nascent pre-mRNA, and more specifically intronic m^6A has been linked to exon skipping events. The occurrence of impaired alternative splicing (AS) is frequently found during the development of cancer. We performed transcriptome wide analysis in breast cancer cell lines and explored AS events. Our results define an AS signature for breast tumorigenesis. We found that METTL3 modulates AS directly through m^6A deposition at the intron-exon junctions or indirectly by the m^6A deposition in transcripts encoding for splicing factors and transcription factors. In particular, we show that *MYC* mRNA harbours the m^6A mark, suggesting that METTL3 regulates AS indirectly *via* the regulation of MYC expression. Indeed, the targets of MYC overlapped with METTL3-associated AS events. Importantly, five of the AS events identified and validated *in vitro*, are linked to a worse prognosis in breast cancer patients. Additionally, we show that METTL3 enhances the breast cancer phenotype through a dual mechanism depending on its sub-cellular localization. We find that the canonical nuclear function of METTL3 decorates transcripts that are involved in cell proliferation and migration. We observe that METTL3 is highly expressed in the cytoplasmic compartment of breast cancer cells from patients. Remarkably, we uncover that cytoplasmic METTL3 interacts with subunits of the exocyst, whose subunit EXOC7 has been linked to cell adhesion, migration and invasion. Notably, we show that breast cancer cell lines depleted of METTL3 display less gelatinase activity and invadopodia formation, supporting the role of METTL3 in cell invasion *via* exocytosis.

m^6A is a reversible modification, which can be demethylated by the erasers FTO and ALKBH5. Depletion of FTO has been shown to increase the level of m^6A in mRNA, however recent studies have reported that FTO could demethylate N^6,2´-O-dimethyladenosine (m^6A_m), adjacent to the 7-methylguanosine cap on mRNA. In the cellular model of colorectal cancer CRC1, depletion of FTO leads to a cancer stem cell phenotype and confers chemotherapy resistance. By performing m^6A-RNA immunoprecipitation followed by sequencing (MeRIP-seq), we show that knockdown of *FTO* in CRC1 cells does not affect the global level of m^6A in mRNA but of m^6A_m level.

Keywords
RNA modification, METTL3, m^6A, alternative splicing, exocyst, breast cancer

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