Epitranscriptomic modifications are changes in the chemical composition of ribonucleic acid (RNA) that affect the fate and function of the modified RNA molecules. One of the most common epitranscriptomic modifications is N6-methyladenosine (m6A), which was first identified in messenger RNA (mRNA) molecules in 1974. The m6A modification is dynamic and reversible. The installation of m6A is mediated by complex of enzymes called m6A 'writers' including METTL3, METTL14 and WTAP proteins. So far, two proteins (FTO and ALKBH5) responsible for the removal of m6A modifications from RNA molecules have also been identified and they are called m6A 'erasers'. Another important group of m6A regulators are m6A 'readers', which recognize m6A modification and affect the role of modified transcripts.

Recent studies have shown that, in addition to being present in mRNA, m6A is also present in non-coding RNAs (ncRNAs) which are RNA molecules without protein coding potential. Long noncoding RNAs (lncRNAs) constitute a class of ncRNAs that are longer than 200 nucleotides.

They play an important role in gene expression regulation. In addition, they are linked with human diseases, including cancer. Although the growing number of reports indicating the relationship between m6A and lncRNAs in the formation and progression of cancer, this exploration has mainly been carried out globally, through the silencing of the m6A writers, erasers, or readers. Moreover, most of the techniques used to determine the localization of m6A sites in RNA are based on the use of antibodies and have serious limitations such as poor reproducibility or resolution. Consequently, the functions of single m6A sites within lncRNAs are very poorly understood.

The aim of this project is to determine the exact location of m6A modifications within selected lncRNAs associated with carcinogenesis and functional characterization of these identified m6A sites. The project will use an innovative, antibody-independent approach to map m6A sites down to one nucleotide. This task will be carried out using breast cell lines as well as RNA extracted from tissues derived from breast cancer patients. The next step will be quantifying the identified modifications, on the basis of which several m6A sites will be selected for further functional analysis, carried out using human breast cancer cell lines.

The results of this project will contribute to expanding knowledge on: epitranscriptomic modifications within lncRNAs related to the process of carcinogenesis; the influence of single m6A sites on the biology of modified lncRNAs; the impact of m6A modifications within the studied long non-coding RNAs on the process of tumor development and progression.