Description for the general public

The term “epigenetics” refers to heritable alterations that are not due to changes in DNA sequence. Epigenetic modifications, such as DNA methylation and histone modification, alter DNA accessibility and chromatin structure, thereby regulating gene expression patterns. The best characterized DNA epigenetic modification in mammals is methylation of the cytosine, which forms the 5-methylcytosine (5-mCyt). Methylation can also occur on the other nucleotides. The methylation of deoxyadenosines has been identified and is a well-described epigenetic feature in prokaryotes. Formation of N6-metyl-2’-deoxyadenosine (6-mdA) plays a role in various biological pathways in bacteria: host-pathogen interactions, DNA replication, nucleotide segregation, mismatch repair, transcription, and translation. DNA 6-mdA modification was considered absent in eukaryotes, including humans, since it was not detectable in earlier generations of studies. Recently 6-mdA was found to be present in lower eukaryotes, vertebrates, and mammals. Few recent studies reported the presence of this modification in human tissue.

Aberrant epigenetic landscapes promote tumor initiation and progression. The focus of cancer research has been on the global and local aberrations of 5-mCyt levels. Genome wide DNA 5-mCyt hypomethylation and promoter specific 5-mCyt hypermethylation may contribute to cancer formation, and these alterations coexist in most, if not all, human cancers. The role of other methylated nucleobases in cancer remains obscure. Only a few studies analyzed 6-mdA levels in human cancer. The level of this modification was significantly decreased in gastric, liver, lung, and breast cancers tissues compared to normal tissues. Interestingly, the opposite tendencies were observed in esophageal squamous cell carcinoma, hepatocellular carcinoma, tongue squamous cell carcinoma, and glioblastoma (cancer tissues - higher 6-mdA levels). However, latest study showed that the content of this modification significantly decreases in all classified gliomas compared with normal brain tissues. Another characteristic feature of malignant cells is a profound decrease in the level of 5-hydroxymethylcytosine, a product of 5-mCyt demethylation. Interestingly, the recently published study reports the presence of N6-(hydroxymethyl)-2’-deoxyadenosine (6-hmdA) in mammalian DNA. The level of this modification was significantly increased in lung carcinoma tissues compared to tumor-adjacent normal tissues.

N6-methyladenosine (6-mrA), the most abundant internal modification in eukaryotic messenger RNAs (mRNAs), has been shown to play vital roles in many normal life processes. Modifications of 6-mrA methylation are dynamic and reversible in mammalian cells and have been proposed as another layer of epigenetic regulation similar to DNA methylation and histone modifications. The 6-mrA mark in mRNA is deposited by the 6-mrA methyltransferase complex (composed of METTL3, METTL14, and WTAP) and can be removed by 6-mrA demethylases such as FTO and ALKBH5. 6-mrA RNA methylation is involved in all stages of the life cycle of RNA. Dysregulation of 6-mrA modification and the associated proteins also contributes to the initiation, progression, metastasis, and drug response of various cancers (for example hematological malignancies).

Therefore, the main objective of this project is to search for the biological role of N6-methyladenine and its derivatives. Processes of methylation and demethylation of adenine derivatives share catalytic mechanisms with methylation/demethylation of cytosine. We hypothesize that these modifications in cancer may undergo similar changes to those observed with 5-mCyt and its oxidation products. For this reason, N6-methyladenine and its derivatives may play a role in hematological malignancies development. To determine the abovementioned modifications in nucleic acids we will use a highly advanced technique, namely automatic online two-dimensional ultra-high performance liquid chromatography with tandem mass spectrometry (2D-UPLC-MS/MS). This method allows us reliable quantification of N6-methyladenine and its derivatives in material from patients with leukemias (acute myeloid leukemia – AML, chronic lymphocytic leukemia – CLL), multiple myeloma (MM) as well as in the control group. We will also analyze material from individuals with myelodysplastic syndrome (MDS) (which may develop into AML), patients with monoclonal gammopathy of undefined significance (MGUS) (almost always precedes MM), as well as patients with premalignant monoclonal B cell lymphocytosis (MBL) with greatest risk of developing full blown CLL. The results of our study may contribute to a better understanding of adenine methylation/demethylation processes and the role of N6-methyladenine and its derivatives in cancer development. Furthermore, our results may provide a background for developing relatively simple and inexpensive laboratory tests, and the determination of the level of N6-methylated adenine derivatives could, in the future, become a standard diagnostic tool in personalized medicine.