Regulation of the Cellular Response to Hypoxia by *N*^{6}-methyladenosine

Theodora Sideri[1], Ilias Mylonis[2], Georgia Chachami[2], Panagiotis Liakos[2], George Simos[2], Folkert van Werven[1]

[1] The Francis Crick Institute, London, NW1 1AT, UK

[2] Laboratory of Biochemistry, Faculty of Medicine, University of Thessaly, 41500, Larissa, Greece

N^6-methyladenosine (or m^{6}A) is the methylation on the sixth position of the nitrogen of adenosine, it is the most widespread internal modification on mRNA, important in embryonic development in mammals, conserved in eukaryotes and found in 1/3 of mammalian mRNAs, with an average of 3-5 m^{6}A modifications per mRNA. It can be deposited by writer (methylases), removed by eraser (demethylases) and bound by reader (RNA-binding) proteins. *N*^{6}-methyladenosine controls mRNA decay and translation, is implicated in several pathologies including cancer exhibiting pro- and less often anti-oncogenic properties although most of the time functions as an oncogene and results in resistance to chemotherapy. Another important aspect of tumours is the development of hypoxic regions and response to hypoxia results in cancer cell survival. The mRNA of the master regulator of the transcriptional response to hypoxia, HIF-1α, is often found decorated by m^{6}A in genome-wide datasets of cancer cell lines. Several more indications in the literature implicate m^{6}A machinery players in hypoxia and cancer but a comprehensive study is lacking. We have developed an ELISA (1) to quantify the modification and find that m^{6}A is elevated in Huh7 cells exposed to hypoxia (1% O_2) compared to normal oxygen conditions. We find that ALBKH5 demethylase expression is increased in HeLa and MCF7 hypoxic cultures and we are investigating further the mechanism. We are also setting up evolved TadA assisted *N*^{6}-methyladenosine sequencing (eTAMseq) (2) in order to generate methylome maps and we will be investigating how m^{6}A regulates mRNA life during hypoxia in cancer cell lines. Ultimately, gaining a mechanistic understanding of the interplays between m^{6}A and hypoxia could lead to better therapeutics and exploit these findings for better drug targets.

1. Ensinck I, Sideri T, Modic M, Capitanchik C, Vivori C, Toolan-Kerr P, van Werven FJ. m6A-ELISA, a simple method for quantifying *N6*-methyladenosine from mRNA populations. **RNA**. 2023 May;29(5):705-712. doi: 10.1261/rna.079554.122.

2. **Xiao YL, Liu S, Ge R, Wu Y, He C, Chen M, Tang W**. Transcriptome-wide profiling and quantification of N^6-methyladenosine by enzyme-assisted adenosine deamination. **Nat Biotechnol**. 2023 Jul;41(7):993-1003. doi: 10.1038/s41587-022-01587-6.