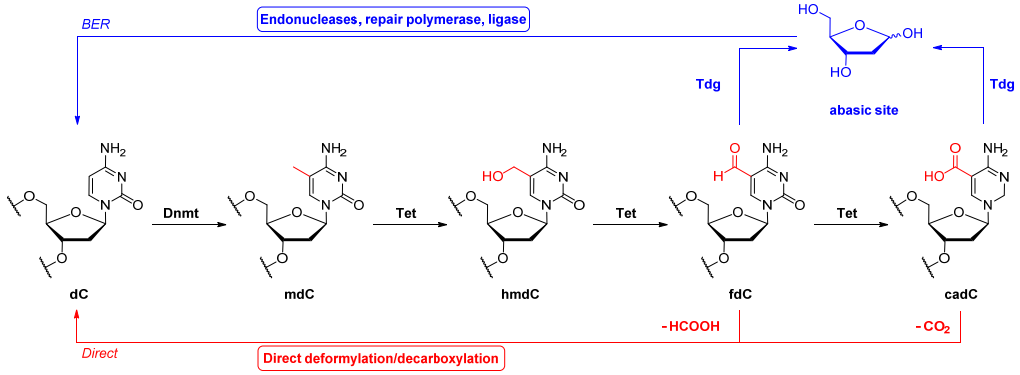
INVESTIGATION OF ACTIVE DEMETHYLATION OF 5-METHYL-2'-DEOXYCYTIDINE

Eva Korytiaková, Angie Kirchner, Katharina Iwan, Sarah Schiffers and Thomas Carell*

Dept. of Chemistry, Ludwig-Maximilians-Universität, Butenandtstr. 5-13, 81377 Munich, Germany

* Eva.Korytiakova@cup.uni-muenchen.de, Thomas.Carell@cup.uni-muenchen.de

Demethylation of 5-methyl-2'-deoxycytidine (mdC) plays an important role during epigenetic reprogramming. Oxidation of mdC catalyzed by ten eleven translocation (Tet) enzymes leads to 5-hydroxy-2'-deoxycytidine (hmdC),[1-2] 5-formyl-2'-deoxycytidine (fdC) and 5-carboxy-2'-deoxycytidine (cadC).[3-5] Discovery of these potentially epigenetic relevant bases led to the idea of two possible active demethylation mechansims: a base excision repair (BER) associated pathway and a direct C-C bond cleavage in form of deformylation of fdC or decarboxylation of cadC.[4,6-7] To investigate the direct cleavage of the C-C bond *in vivo*, 5-substituted chemically modified cytidine derivatives were synthesized and incorporated into the genome of stem cells. The incorporated nucleosides can undergo biochemical changes, which can be analyzed after enzymatic digestion of DNA with UHPLC-triple-quadrupole MS techniques. Analysis of the isolated nucleosides give crucial hints towards one of the mechanisms of active demethylation of mdC.



To study the base excision mediated repair mechanism, the analysis and quantification of during this process cleaved nucleobases is required. For MS quantification methods, new chemoselective reagents were developed, that specifically target the cleaved bases. These derivatization reagents and newly developed analytical protocols allow the investigation of the BER mechanism in deeper detail.

Acknowledgment: This work was supported by Fonds der Chemischen Industrie through a Kekulé-Stipendium, the Deutsche Forschungsgemeinschaft and the Excellence Cluster CiPS^{M})

[1] Tahiliani et al. Science **2009**, 324, 930-935.

[2] Kriaucionis et al. Science **2009**, 324, 929-930.

[3] Ito et al. Science **2011**, 333, 1300-1303.

[4] He et al. Science **2011**, 333, 1303-1307.

[5] Pfaffeneder et al. Angew. Chem . Int. Edit. **2011**, 50, 7008-7012.

[6] Schiesser et al. J. Am. Chem. Soc. **2013**, 135, 14593-14599.

[7] Maiti et al. J. Biol. Chem. **2011**, 286, 35334-35338.