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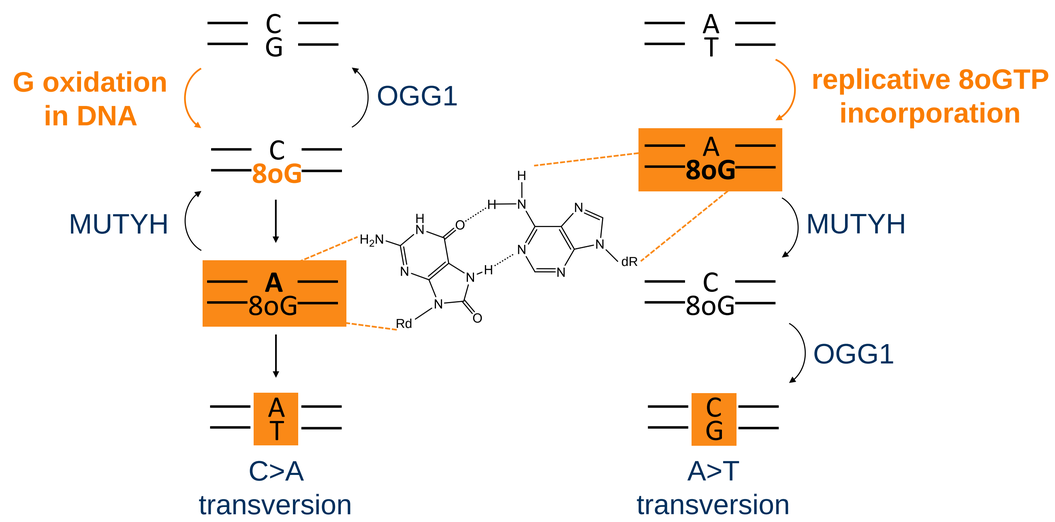
Assessment of potentially divergent pathways for processing of the pre-mutagenic 8oG:A lesion

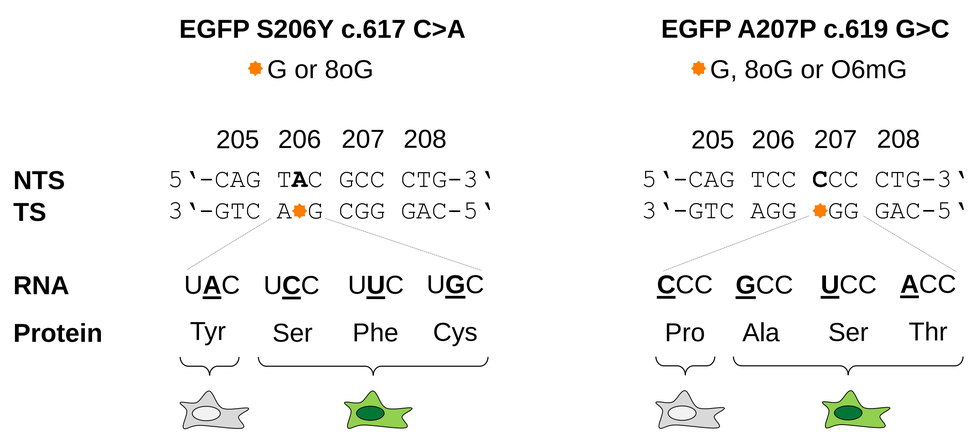
BACKGROUND: THE MUTAGENICITY OF 8OG:A



Depending on the source of 8-oxo-7,8-dihydroguanine (8oG) in the genome, the MUTYH pathway can either prevent or promote the mutagenicity. We are therefore seeking a complementary pathway for processing of the pre-mutagenic 8oG:A lesion.

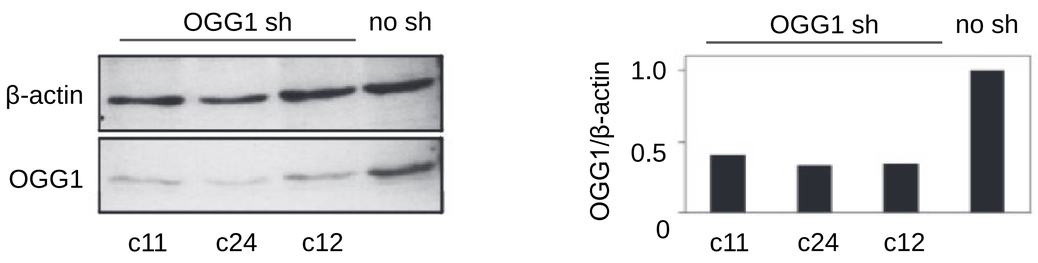
THE TRANSCRIPTIONAL MISCODING (TM) ASSAY

We adopted two EGFP loss-of-fluorescence mutants to investigate the of repair of 8oG and O6mG, DNA lesions with pronounced miscoding properties, by incorporating them at the selected nucleotide positions.



2 SENSITIVE DETECTION OF REPAIR OF 8OG

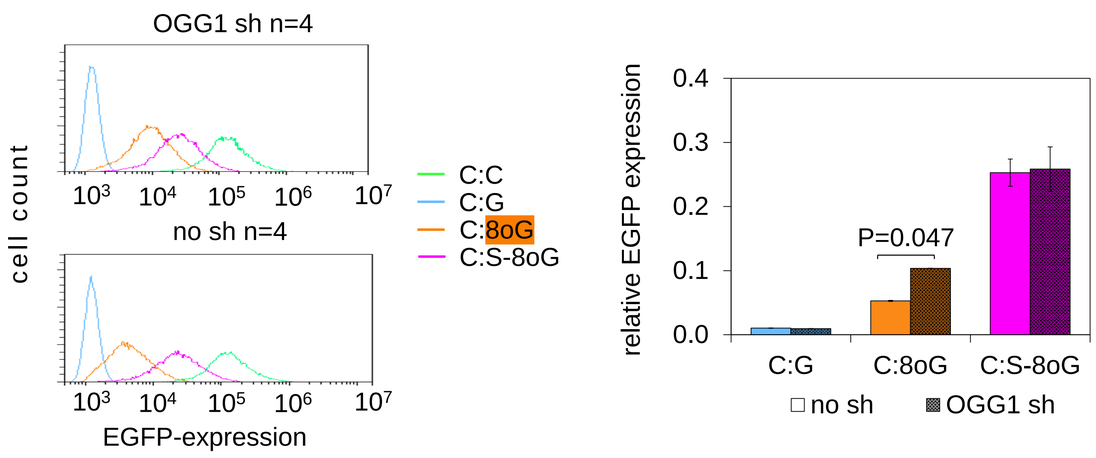
Stable OGG1 knockdown in HeLa cells (OGG1 sh)



Allgayer et al. (2013), NAR

Analysis of the EGFP-expression of the mutant A207P c.619 G>C carrying 8oG and S-8oG. The constructs were transfected to Hela pEps cells (no sh) and HeLa pEps OGG1 sh (c12) cells.

8oG:C causes TM, which is increased 2-fold in OGG1-depleted cells and 5-fold when the BER-resistant analog (S-8oG) was used.



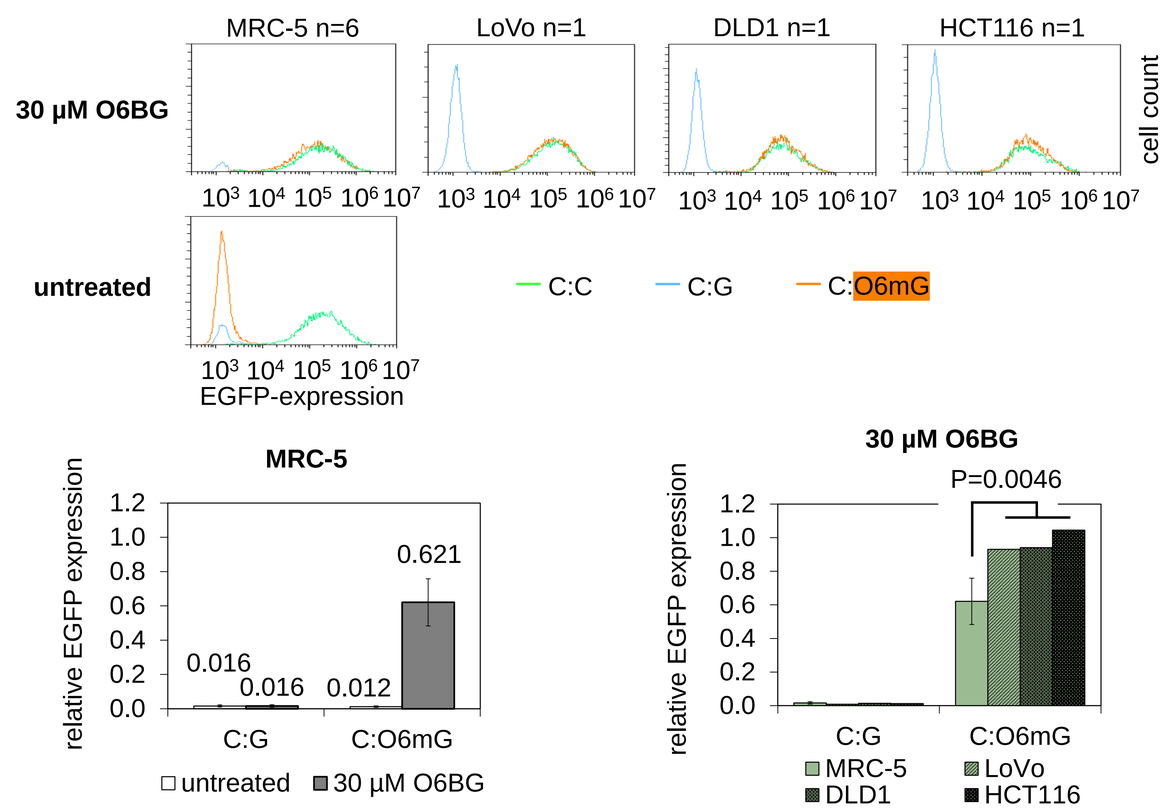
KEY FINDINGS

- DNA modifications and their repair can be efficiently detected by a reporter assay based on transcriptional miscoding (TM) [1]
- Repair analysis of O^6-methylguanine (O6mG) suggested that MMR can contribute to repair of miscoding DNA lesions in principle [1]
- 8oG can be efficiently detected with the TM approach [2]
- In the case of 8oG opposite to C, the mutation rate is lowered by the OGG1-dependent repair [2]
- 8oG opposite to A is also efficiently detected by the TM analysis, but is not removed by OGG1 [3]
- In summary, the results provide scientific rationale and methodology for investigation of potential role of MMR in the processing of the 8oG:A lesion

1 REPAIR OF O6MG AND THE ROLE OF MMR

Analysis of the EGFP-expression of the mutant A207P c.619 G>C carrying a synthetic O6mG. The constructs were transfected to MRC-5 cells mismatch repair (MMR)-proficient and three MMR-deficient cell lines: LoVo (lacking MSH2), DLD1 (lacking MSH6), HCT116 (lacking MLH1) in the presence of the MGMT inhibitor O^6-Benzylguanine

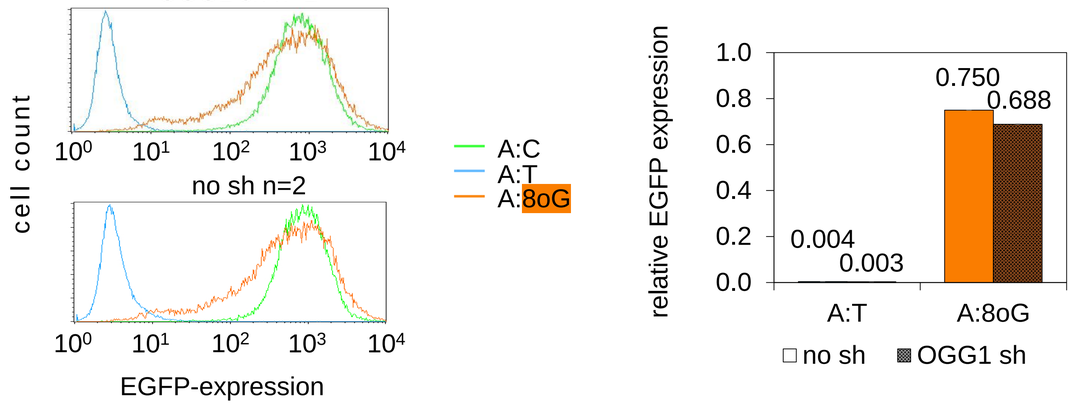
O6mG:C leads to high TM and recovery of fluorescence in MGMT-deficient cells. In MMR-deficient cells, O6mG leads to a significantly higher fluorescence than in cells being capable of mismatch repair.



3 OGG1 DOES NOT REPAIR 8OG:A

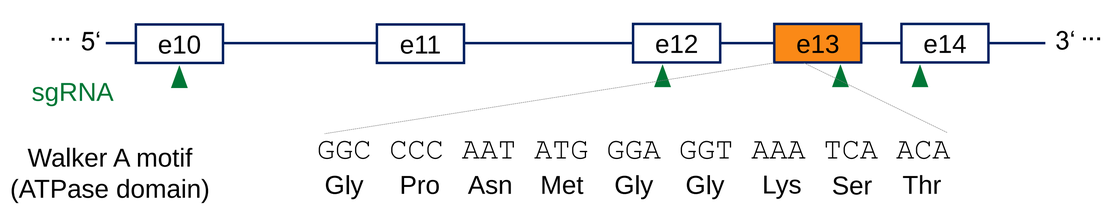
Analysis of the EGFP-expression of the mutant S206Y c.617 C>A 8oG. The constructs were transfected to Hela pEps cells (no sh) and HeLa pEps OGG1 sh (c12) cells.

8oG:A leads to high TM. The relative EGFP-expression in OGG1-deficient cells does not differ significantly from the EGFP-expression in cells expressing OGG1.



OUTLOOK

Considering that MMR removes O6mG, it is plausible that it may also recognise the non-Watson-Crick 8oG:A pair. Therefore, we are generating an MSH2-knockout in HeLa cells to investigate MMR impact on the repair in an isogenic cell model.



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FUNDING

DFG Deutsche Forschungsgemeinschaft