Assessment of Nucleotide Excision Repair of 8-Oxoguanine by a New Transcriptional Mutagenesis Assay

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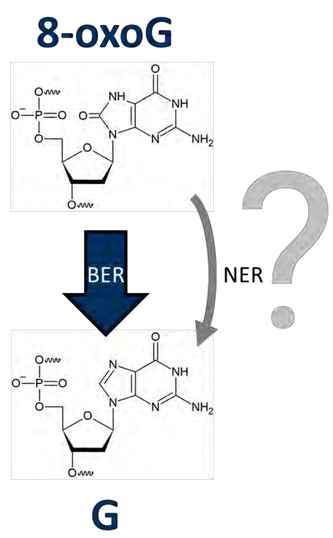
Aim

Background:

- 8-Oxo-7,8-dihydroguanine (8-oxoG) is one of the most common oxidation products in DNA
- 8-OxoG is premutagenic by forming non-canonical base pairs with adenine during DNA replication
- Similarly, it is miscoding during transcription, leading to transcriptional mutagenesis (TM)
- Base excision repair (BER), initiated by OGG1, protects from mutations by efficiently removing 8-oxoG
- In addition, evidence emerges that other pathways might contribute to the repair of 8-oxoG

Our aim was to investigate the putative role of nucleotide excision repair (NER) as an alternative pathway in the repair of 8-oxoG.



Key Findings and Outlook

⇒ We developed a reporter assay to detect 8-oxoG using TM as a readout

⇒ Measurable levels of TM were detected in repair proficient cell lines, demonstrating the sensitivity of the assay **(Fig. 1)**

⇒ Synthetic BER resistant 8-oxoG analog displayed higher TM rates than BER substrate 8-oxoG **(Fig. 1)**

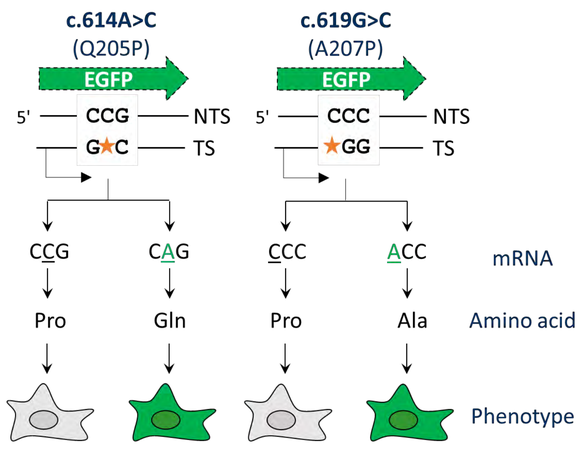
⇒ TM was inversely proportional to the BER capacity of the host cells, proven by both knockdown and knockout of OGG1 **(Fig. 2 and 3)**

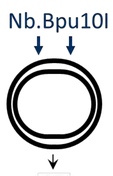
⇒ Cells with higher NER proficiency displayed reduced TM by 8-oxoG suggesting an involvement of NER in 8-oxoG repair **(Fig. 4)**

⇒ Isogenic CRISPR/Cas9 knockouts of XPC (GG-NER) and XPA (NER) in HeLa cells are currently being generated to prove the role of NER in 8-oxoG repair

Experimental Strategy

We incorporated synthetic 8-oxoG, S-8-oxoG or G into the transcribed strand (TS) of the loss-of-function *EGFP* mutant reporters. At the chosen positions, misincorporation of adenine in the course of transcriptional mutagenesis would lead to reactivation of the fluorescent EGFP protein.

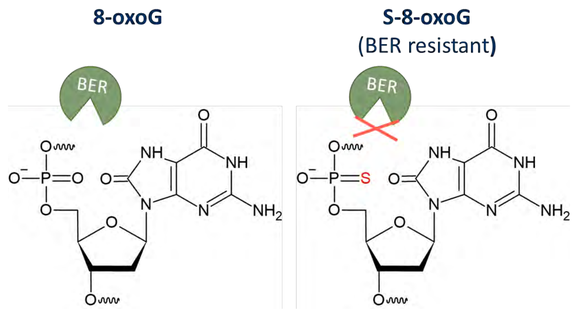




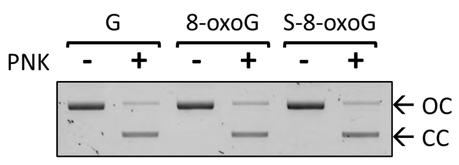
Double nick of the reporter plasmid

Gap generation using an oligonucleotide complementary to the nicked sequence

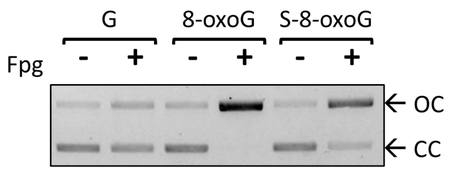
Ligation of the gapped plasmid with a **synthetic oligonucleotide** containing guanine or a single base modification in the form of one of the following lesions:



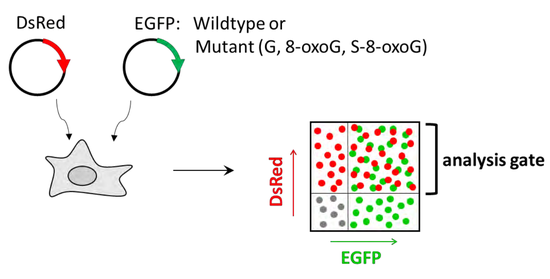
Ligation with and without PNK demonstrated that synthetic oligonucleotides with indicated modifications were efficiently incorporated into vector DNA. Arrows indicate the open circular (OC) and covalently closed (CC) forms.



Cleavage with Fpg confirmed the presence of 8-oxoG. The Fpg activity was slightly inhibited by the phosphorothioate linkage (S-8-oxoG).

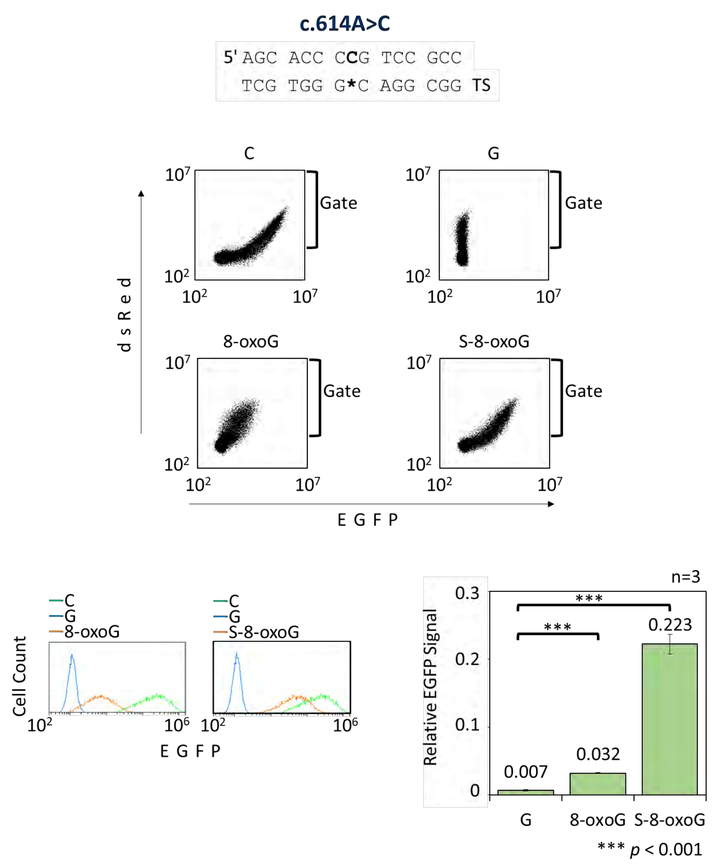


Quantitative expression analyses (FACS) in transfected host cells were performed to measure the TM rates.



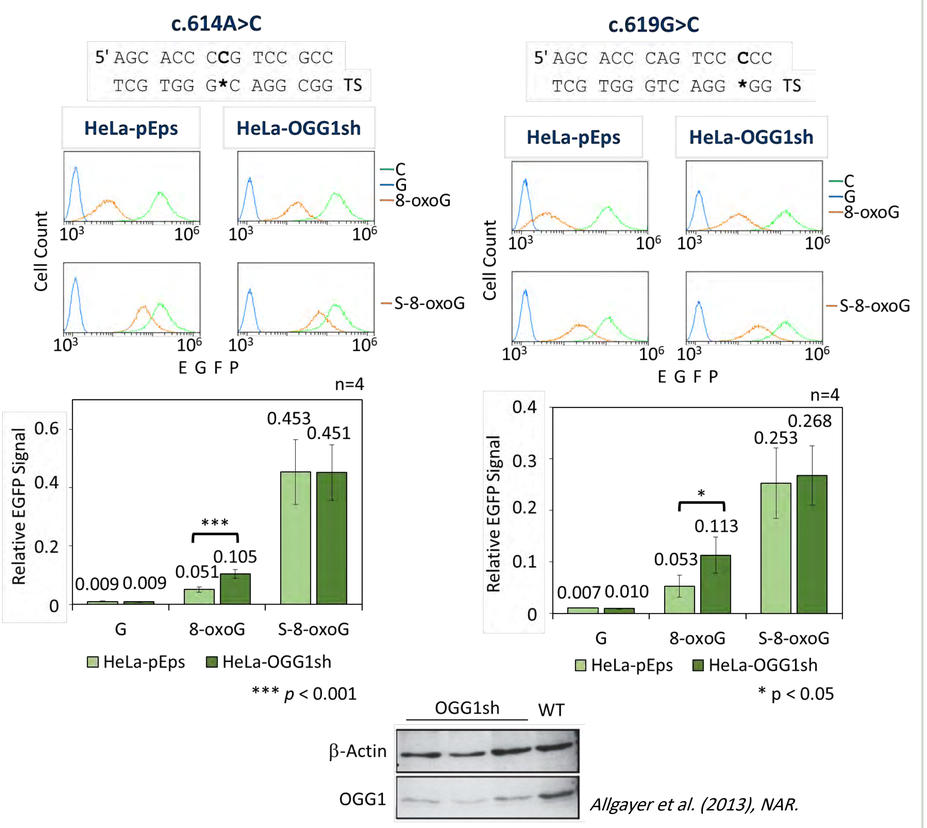
Results

1 8-OxoG Leads to Transcriptional Mutagenesis



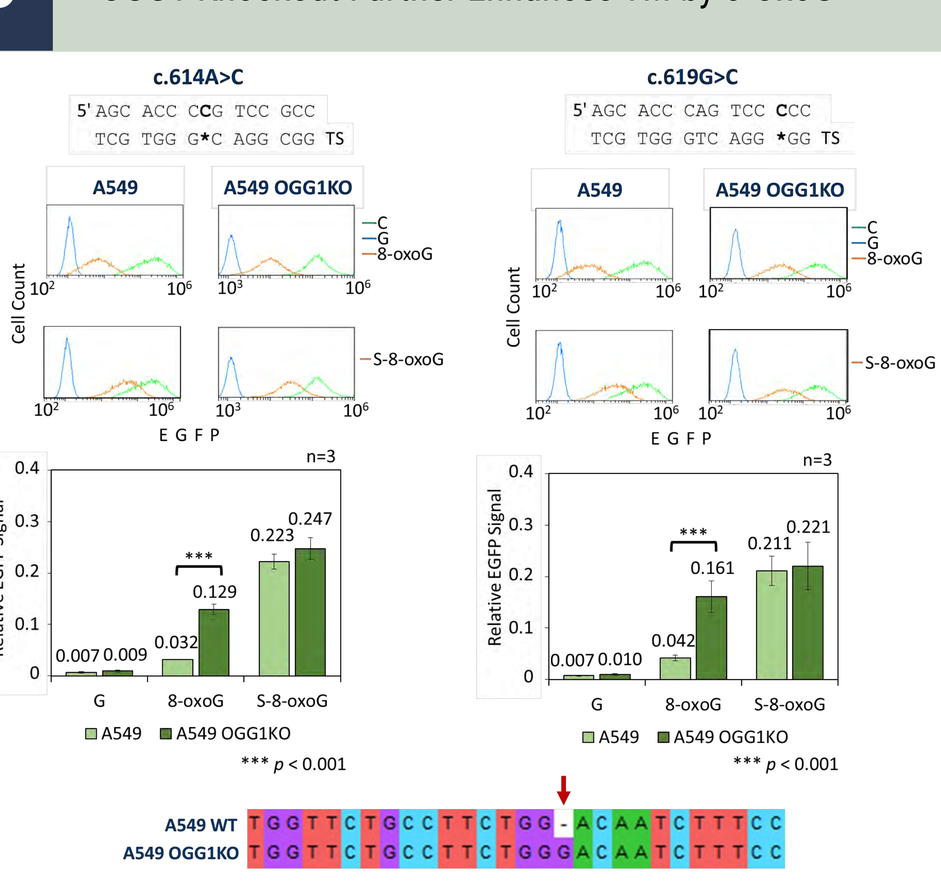
EGFP reactivation by TM was measured 24 h after transfection of A549 cells with expression constructs harboring G, 8-oxoG or S-8-oxoG at the nucleotide 614 in the transcribed DNA strand (TS). TM is caused by 8-oxoG and further enhanced when BER is inhibited by the phosphorothioate 5′ linkage (S-8-oxoG).

2 OGG1 Knockdown Increases TM by 8-OxoG



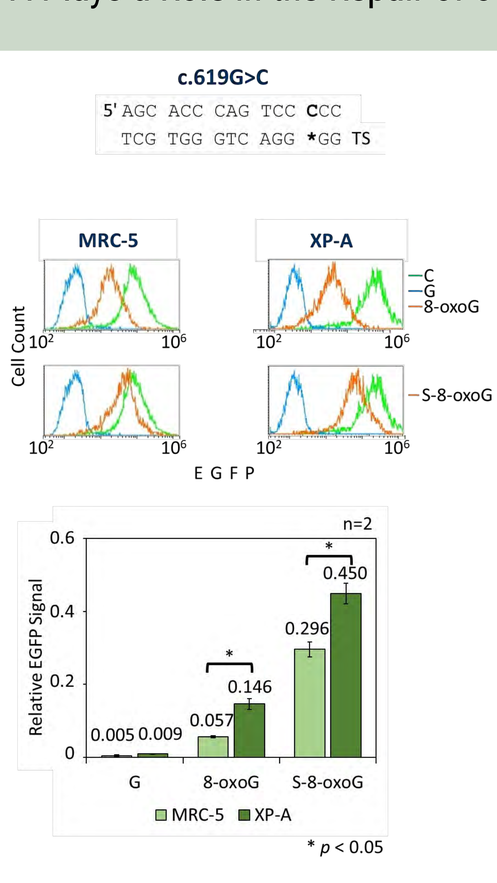
EGFP reactivation by TM was measured 24 h after transfection of plasmids harboring G, 8-oxoG or S-8-oxoG at position 614 or 619. OGG1 knockdown in HeLa cells (*HeLa-OGG1sh*) causes doubled TM rates by 8-oxoG compared to the empty vector control (*HeLa-pEps*). TM caused by S-8-oxoG is considerably higher than 8-oxoG and remains unaffected by OGG1 knockdown. Western blot illustrates the decreased protein expression of OGG1 after knockdown using shRNA expressing vectors.

3 OGG1 Knockout Further Enhances TM by 8-OxoG



EGFP reactivation by TM was measured 24 h after transfection of plasmids harboring G, 8-oxoG or S-8-oxoG at position 614 or 619. OGG1 knockout in A549 cells causes TM rates four times as high as the isogenic wildtype counterpart, which proves the notion of suppression of TM by BER. TM caused by S-8-oxoG is considerably higher than 8-oxoG and remains unaffected by OGG1 knockout. The sequence alignment shows a validation of CRISPR/Cas9 knockout based on an insert in the *OGG1* gene of A549 cells.

4 XPA Plays a Role in the Repair of 8-OxoG



EGFP reactivation by TM was measured 24 h after transfection of plasmids harboring G, 8-oxoG or S-8-oxoG at position 619. XP-A cells (GM04312) derived from Xeroderma pigmentosum patients have impaired NER deficiency and show TM by 8-oxoG twice as high as NER proficient MRC-5 fibroblasts. This indicates an involvement of XPA in the repair of 8-oxoG.