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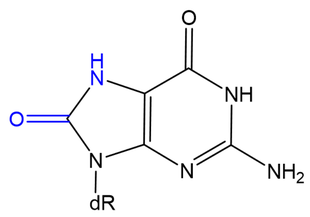
Investigating alternative repair pathways of 8-oxo-7,8-dihydroguanine via transcriptional mutagenesis assay

Background

Base excision repair (BER), initiated by the DNA N-glycosylase OGG1, is the primary pathway that removes 8-oxo-7,8-dihydroguanine (8-oxoG) from the genome. However, mutation data from the *Ogg1*$^{-/-}$ mice suggest a presence of some backup repair (Minowa et al. 2000, PNAS, 97: 4156). Functional interconnections were reported between the mammalian excision repair pathways, which suggested involvement of the nucleotide excision repair (NER) components in the response to oxidatively generated damage (Kumar et al. 2020, NAR, 48: 11227). We have recently developed reporters for a highly sensitive detection of repair of structurally defined DNA lesions, based on their abilities to induce transcriptional mutagenesis (TM) (Rodriguez-Alvarez et al. 2020, Biomolecules, 10: 902). This is a promising approach to resolve the long standing question about relevance of NER to the repair of 8-oxoG in human cells.



Aim

We aimed at the assessment of the repair capacity of human cells deficient in the specific NER components towards 8-oxoG

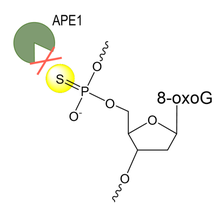
Key Findings and Outlook

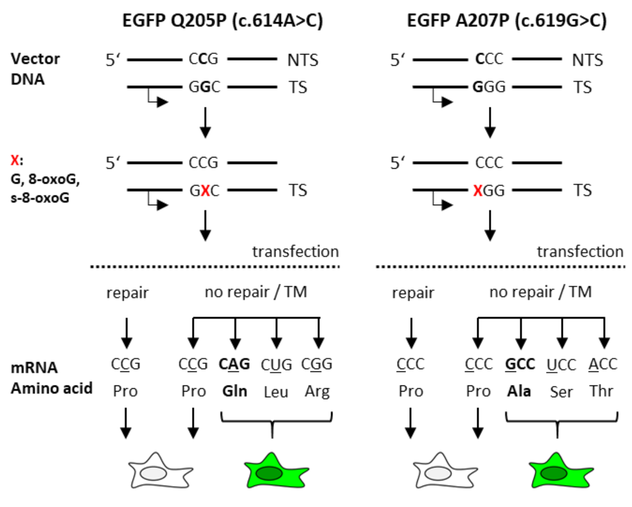
- **TM-based reporter assay is suitable to detect 8-oxoG repair (1)**
- **Synthetic BER-resistant 8-oxoG analog display higher TM rates than the natural lesion (1)**
- **TM is inversely proportional to the BER capacity of the host cells, as proven by knockout of OGG1 (2)**
- **NER-deficient XP-A cells show elevated TM rates by 8-oxoG suggesting an involvement of NER in the repair of 8-oxoG (3)**
- **Surprisingly DDB2, CSA, or XPC knockouts do not strongly influence the repair of 8-oxoG, as judged by the TM rates (4)**
- **CRISPR/Cas9 XPA knockout in HeLa cells shall scrutinize the role of NER in an isogenic pair of cell lines**

Results

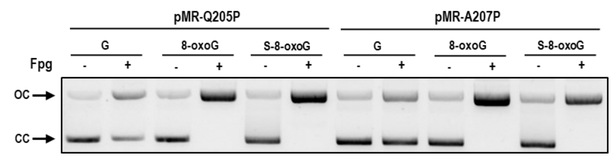
1 Detection of 8-oxoG repair based on suppression of transcriptional mutagenesis (TM)

1. The principle of the reporter assay is the detection of TM by guanine modification. Synthetic guanine (G), 8-oxo-7,8-dihydroguanine (8-oxoG), or its BER-resistant analog with 5'-phosphorothioate linkage (s-8-oxoG) were incorporated in the specific position of the *EGFP* mutant transcribed strand (TS).

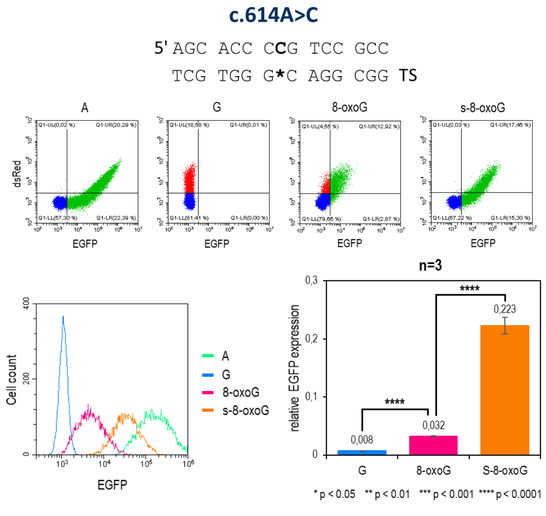




2. **Presence of 8-oxoG or s-8-oxoG,** as confirmed by cleavage with Fpg.

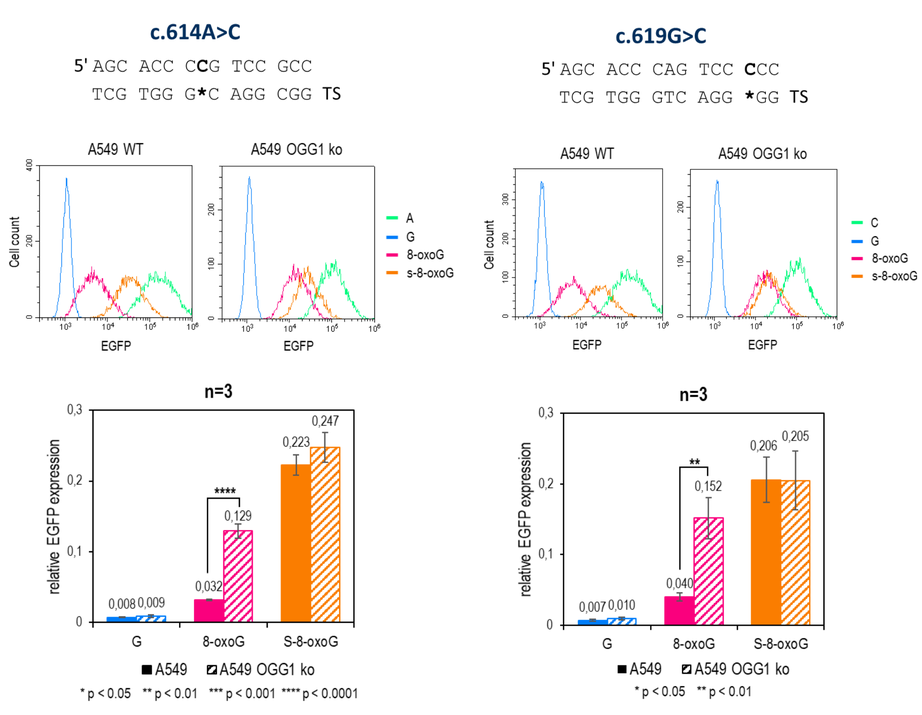


3. **TM by 8-oxoG** measured by flow cytometry in A549 cells transfected with TM reporter contructs containing G, 8-oxoG or s-8-oxoG.



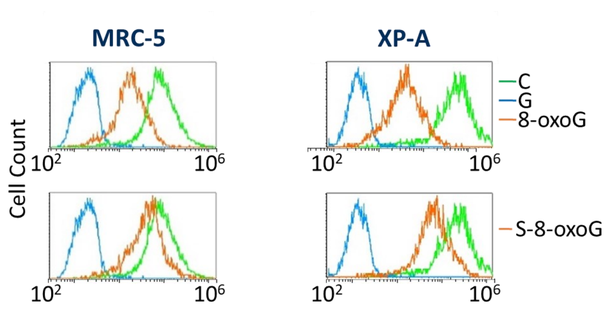
2 OGG1 knockout leads to strong increase of TM through bypass of 8-oxoG

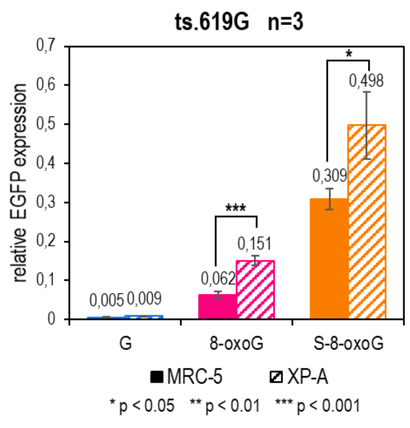
1. Comparison of TM rates of 8-oxoG and S-8-oxoG in A549 OGG1 knockout with A549 WT
2. OGG1 knockout causes TM rates four times as high as the isogenic wildtype counterpart, which proves the notion of suppression of TM by BER
3. TM caused by S-8-oxoG is considerably higher than 8-oxoG and remains unaffected by OGG1 knockout



3 XPA deficiency leads to increased TM by 8-oxoG

NER-deficient XP-A cells (GM04312) show TM by 8-oxoG twice as high as NER proficient MRC-5 fibroblasts, which indicates an involvement of XPA in the repair of 8-oxoG

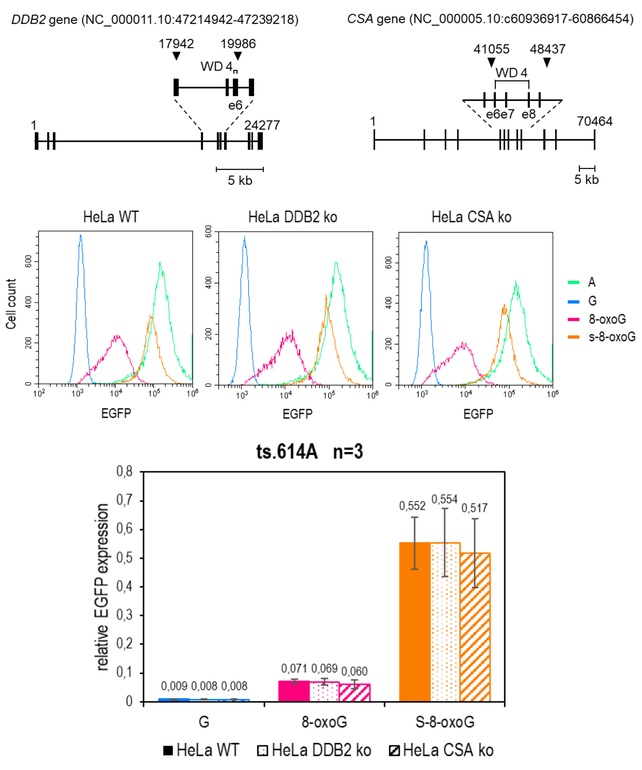


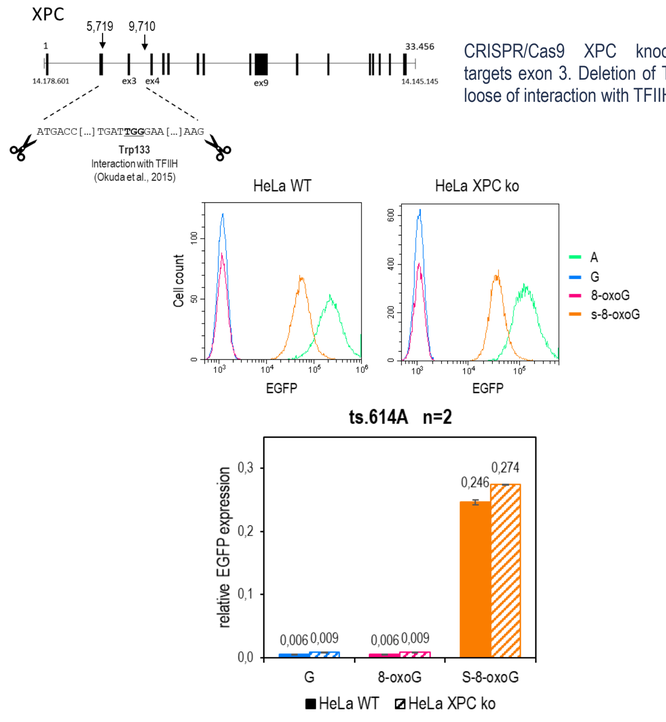


4 Single DDB2 or CSA knockout have no influence while XPC knockout might lead to a slightly increase of TM by 8-oxoG

No significant differences of TM rates caused by 8-oxoG or S-8-oxoG in DDB2 knockout or CSA knockout cells compared to WT, which indicates DDB2 and CSA independent repair pathway

Slight increase of TM rates caused by 8-oxoG or S-8-oxoG in XPC knockout cells compared to WT, which indicates that NER might play a minor role in the repair of 8-oxoG





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