

# Does the modification of serine 477 of DNA mismatch repair protein MLH1 play a role in cell proliferation?

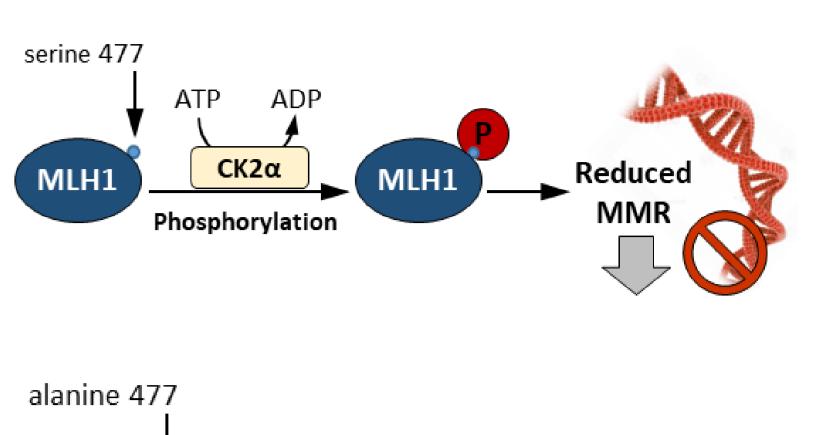
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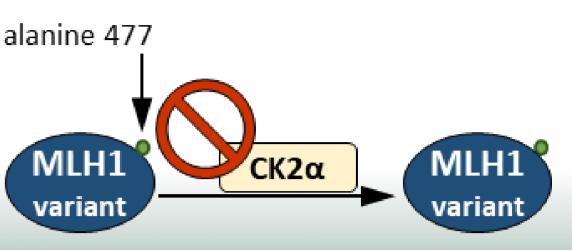
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### Introduction

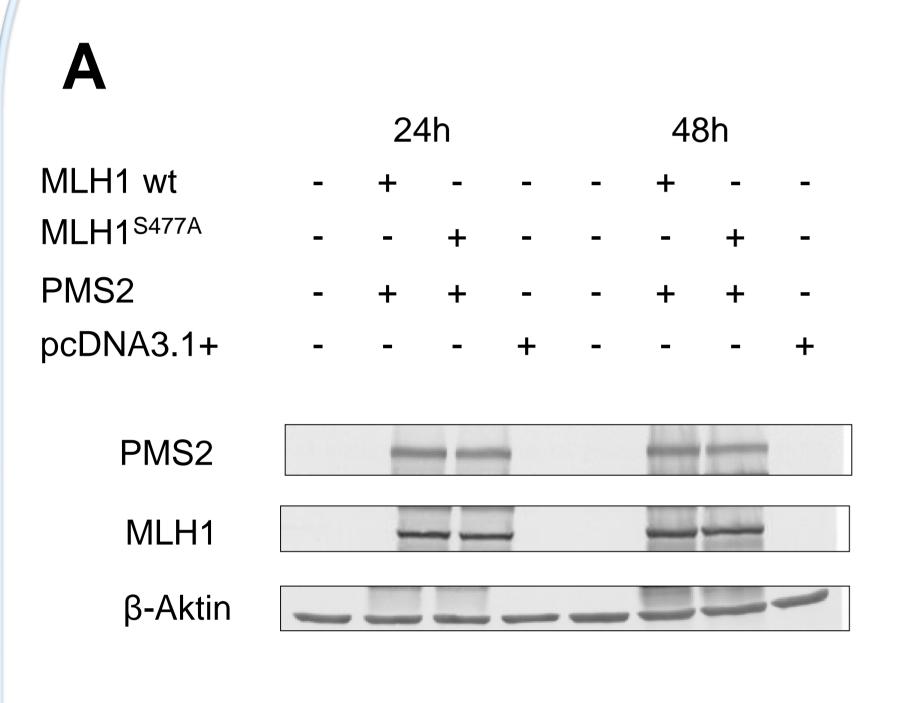
MutL $\alpha$ , a heterodimer consisting of MLH1 and PMS2, is a key player of the DNA mismatch repair (MMR) system and of great importance to correct incorporation errors that occur during DNA replication. Previously, we identified that posttranslational phosphorylation of MLH1 at amino acid position serine 477 can switch off MMR activity in vitro. We also found that mutation of serine 477 prevented the posttranslational phosphorylation.

Since MLH1 is involved in numerous MMR-independent cell processes, including the cell cycle control, we hypothesized that phosphorylation of MLH1 might alter the mediation of cell cycle-associated proteins and thus affects proliferation.

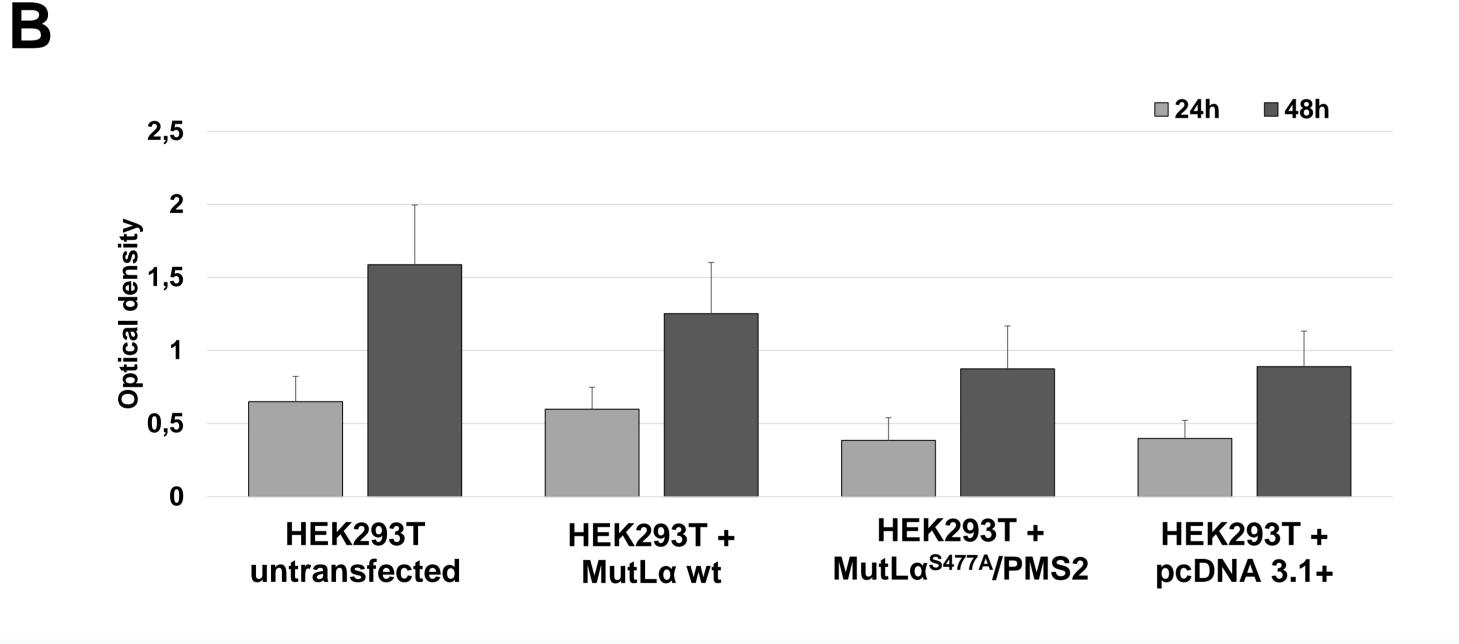




## Investigation of the influence of different transfections on proliferation

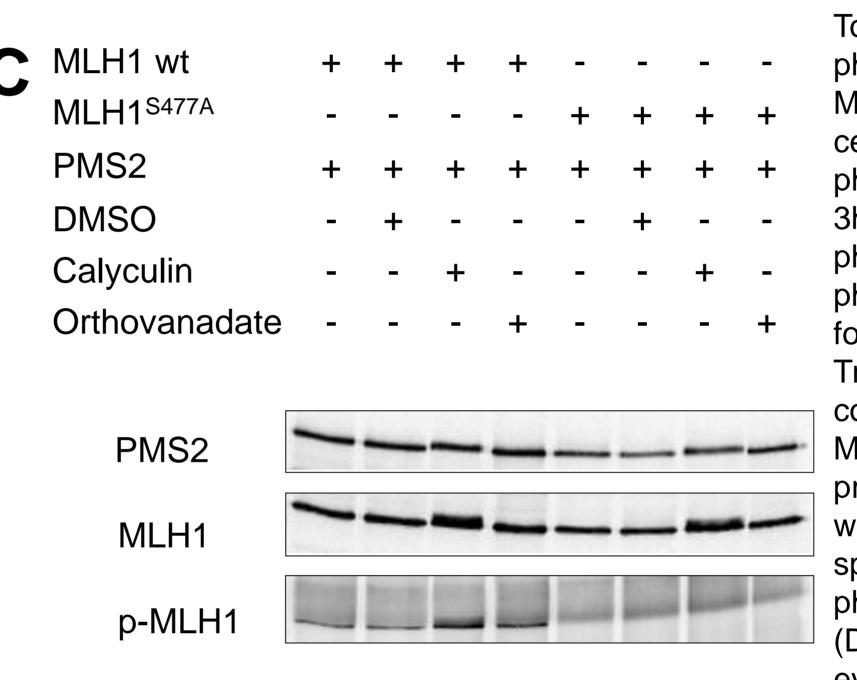


MutLa deficient HEK293T cells were transiently cotransfected pcDNA3.1+/MLH1 and pcDNA3.1+/ PMS2 for the expression of MutLα wildtype. For the expression of the non-phosphorylatable MutLα variant cells were transiently cotransfected with pcDNA3.1+/MLH1<sup>S477A</sup> pcDNA3.1+/PMS2. Non-transfected as well as mock transfected (pcDNA3.1+) HEK293T cells served as controls. After further cultivation periods of 24h and 48h (A) Western blot analysis was performed with 50 µg protein as well as (B) MTT-Assay was carried out evaluated via ELISA reader.

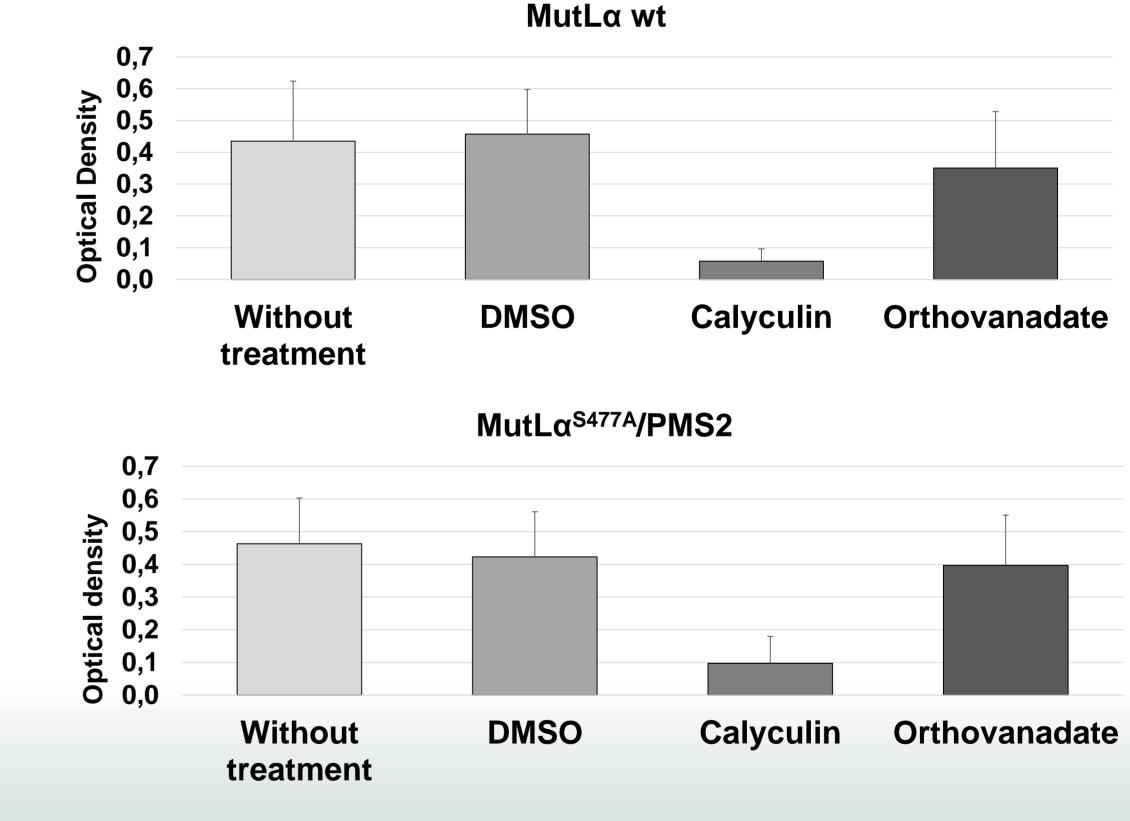


Non-phosphorylatable MLH1<sup>S477A</sup>/PMS2 overexpressing cells show less proliferation than MutLα wildtype overexpressing cells. The phosphorylation of MLH1 seems to play a role in cell proliferation.

### Determination of the effect of phosphorylation of MLH1 on cell proliferation



MLH1 examine impact MutLα phosphorylation, MutLα<sup>S477A</sup>/PMS2 transfected HEK293T cells were treated with the serine/threonine phosphatase inhibitor Calyculin (50nM for 3h) to enhance the amount of phosphorylated MLH1 or with the tyrosine phosphatase inhibitor Pervanadate (50µM for 3h) to exclude treatment side effects. Treatment with DMSO served as negative control. (C) Immunoprecipitation using anti-MLH1 anti-body was completed with 200µg protein and the detection of p-MLH1° was carried out by Western blotting using a specific antibody which recognizes the phospho-S477-motif RHREDS\* of MLH1. (D) In parallel, cell proliferation was evaluated after 48h via MTT-assay.



Non-phosphorylatable MLH1<sup>S477A</sup>/PMS2 overexpressing cells show slightly increased proliferation after Calyculin treatment compared to MutLα wildtype overexpressing cells.

### Conclusion

In summary, significant differences of proliferation could be detected between the differently treated cells. Proliferation of Calyculin treated HEK293T cells overexpressing the non-phosphorylatable MutLα variant, however, was only weakly increased compared to cells overexpressing MutLα wildtype. Due to the fact that Calyculin and Orthovanadate are able to influence a multitude of signaling pathways, the role of MLH1 phosphorylation cannot be conclusively answered here. Further experiments are necessary to clarify the function of phosphorylated MLH1 in proliferation.

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