Structural and functional analysis of the HIRAN domain of human HLTF protein

Ph.D. thesis summary

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Introduction

DNA in our cells is continuously under assault from different damaging agents such as UV irradiation, reactive oxygen species, metabolites, and chemicals. These agents change the structure of the DNA molecule. To avoid these mutations, many DNA repair mechanisms have evolved. These mechanisms are able to restore the original structure of the DNA double helix. Some damages reach the S-phase of the cell cycle where they can cause stalling of the replication fork leading to double-strand breaks, chromosomal rearrangements, and cell death. To avoid these consequences, a DNA damage bypass pathway has evolved which protects the stalled replication fork in different ways.

The main step of the pathway is the monoubiquitylation of the PCNA protein, the processivity factor of polymerases, by the Rad6/Rad18 complex at the lysine 164 position. Following this modification, the replicative polymerase can be switched to an alternative polymerase, which is able to synthesize through the lesion. In another error free mechanism, the monoubiquitylated PCNA becomes polyubiquitylated by the Mms2/Ubc13/HLTF complex through the lysine 63 residue, therefore, HLTF can reverse the replication fork. On this newly emerged so-called chicken foot structure, the stalled strand can be completed using the newly synthesized sister strand as a template. The third possibility is an alternative template switching mechanism.

Our study focuses on the better understanding of the function and regulation of the DNA damage bypass pathway. Our goal is to shed light on the activities and regulatory role of the HLTF protein. HLTF was identified as a transcription factor and tumor suppressor. In a high percentage of colon cancers, the promoter of HLTF is hypermethylated, or a truncated form of the protein is expressed. The known main activities of the HLTF protein were discovered in our research group. The protein plays a highly significant role in the maintenance of genome stability. Reduced expression of the protein leads to the accumulation of DNA mutations and genome instability. The domain structure of HLTF is well known. It has a RING-type ubiquitin ligase domain and helicase domains responsible for dsDNA-dependent translocase activity. However, the function of the HIRAN domain is unknown.

Aims

Our goals were the followings:

1.) To generate HIRAN domain mutant HLTF proteins, and to examine the effect of the mutations.

2.) To test the ligase and helicase activity of the purified proteins to rule out the possible structural mutations.

3.) To investigate whether the HIRAN domain has any effect on the replication fork reversal activity of HLTF protein.

4.) To investigate the *in vivo* function of the mutant proteins via sensitivity assay and comet assay using different DNA-damaging agent.

5.) To purify the HIRAN domain to examine its DNA-binding ability and to specify the preferred DNA structures.

Methods

1. Restriction modification, ligation
2. PCR reaction
3. LR reaction
4. Plasmid cloning
5. Bacteria transformation
6. Plasmid purification from bacteria
7. Yeast transformation
8. Protein purification
9. Polyacrylamide gel electrophoresis
10. Western blot analysis
11. DNA substrate hybridization
12. Replication fork-reversal assay
13. Gel shift and DNA competition assay
14. PCNA ubiquitylation assay
15. ATPase assay
16. *In vivo* protein localization assay
17. BrdU comet assay
18. UV sensitivity assay

Summary of the results

1. Cloning and *in vitro* activity testing of the HIRAN domain mutant HLTF proteins

For the *in vitro* study of HIRAN domain, first with the help of our collaborators we identified the 3D structure of the domain. It turned out from the structure that the HIRAN domain possibly binds DNA. We could also identify the main amino acids that may be responsible for HIRAN to bind DNA. Considering this result we generated a HIRAN deletion mutant and a double point mutant HLTF protein. We overexpressed the proteins in yeast followed by the affinity purification of the mutants. Next we investigated the in vitro activity of the HIRAN mutant proteins.

We tested the known activities of HLTF proteins and showed that the HIRAN mutants have ATPase and double stranded DNA-dependent helicase activity. They can also polyubiquitylate PCNA in the presence of MM2-UBC13 complex just like the wild type HLTF. From these results we concluded that our HIRAN domain mutant HLTF proteins have normal structure, the mutations have impact only on the HIRAN domain. On the other hand, the mutants lost their replication fork-reversal activity. So, from our experiments we concluded, that the HIRAN domain is responsible for the replication fork-reversal activity of the HLTF protein *in vitro*.

2. *In vivo* activity testing of the HIRAN domain mutant HLTF proteins

We investigated the effect of the HIRAN domain mutation on the cellular function of HLTF protein. The HLTF mutant human cells are sensitive to different DNA damaging agents. We showed that after treated with UV irradiation, the HIRAN domain mutant HLTF proteins can be found in the nucleus, however the UV sensitivity of the HIRAN domain mutant HLTF-expressing cells is the same as the sensitivity of the HLTF shRNA depleted cells.

Using BrdU comet assay we showed that in HLTF shRNA depleted cells the replication was completed in longer time compared to the wild type cells. We showed the same effect in the HIRAN domain mutant HLTF-expressing cells. In these cells the replication needed more time to complete than the wild type cells.

From our results we concluded that the HIRAN domain of the HLTF is crucial for all the activities of the protein *in vivo*.

3. DNA binding ability of the HIRAN domain

We overexpressed and purified wild type and double point mutant HIRAN domain from yeast. We showed using gel shift assay that the point mutant domain was not able to bind any DNA substrates. Using the wild type HIRAN domain we

could show interaction with single stranded DNA and replication fork substrate. However HLTF was not able to bind to double stranded DNA. In competition analysis we showed that HIRAN can bind stronger to replication fork, than to single stranded DNA.

The HIRAN domain-deleted HLTF protein has no replication fork-reversal activity. However, mixed with wild type HIRAN domain, activity typical for wild type HLTF could be observed again.

From this and previous results we concluded that the HIRAN domain is responsible for the replication fork-reversal activity of human HLTF protein, and HIRAN domain is essential for all the activities of HLTF *in vivo*. The supposed function of HIRAN domain is to recruit the HLTF protein to the stalled replication fork. After that it helps to bind to the fork, and to reverse it, to promote the restart of the replication.

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List of publications

Publications supporting the dissertation:

Achar Yathish[#], Balogh David[#], Neculai Dante[#], Juhasz Szilvia, Morocz Monika, Gali Himabindu, Dhe-Paganon Sirano, Venclovas Ceslovas, Haracska Lajos. Human HLTF mediates postreplication repair by its HIRAN domain-dependent replication fork remodelling. NUCLEIC ACIDS RESEARCH &: pp. in-press. (2015). **IF: 9.11** MTMT: 2933791

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