

## [4Fe-4S] cluster-containing human exonuclease V acts as a novel replication fork restart factor

Chi-Lin Tsai<sup>a</sup>, Shashank Hambarde<sup>b</sup>, Tej K. Pandita<sup>c</sup>, and John A. Tainer<sup>d,e</sup>

<sup>a</sup>Department of Molecular and Cellular Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA. [CTsai5@mdanderson.org](mailto:CTsai5@mdanderson.org)

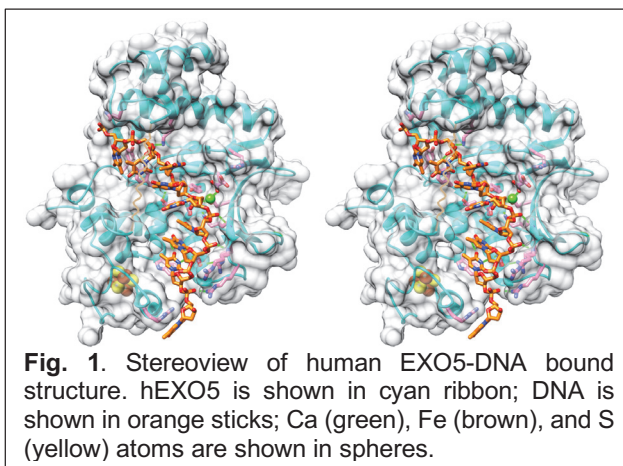
<sup>b</sup>Department of Radiation Oncology, The Houston Methodist Research Institute, Houston, TX 77030, USA. [shambarde@houstonmethodist.org](mailto:shambarde@houstonmethodist.org)

<sup>c</sup>Department of Radiation Oncology, The Houston Methodist Research Institute, Houston, TX 77030, USA. [tpandita@houstonmethodist.org](mailto:tpandita@houstonmethodist.org)

<sup>d</sup>Department of Molecular and Cellular Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA. [JTainer@mdanderson.org](mailto:JTainer@mdanderson.org)

<sup>e</sup>Molecular Biophysics and Integrated Bioimaging, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA.

As most cancer mutations arise from replication, error-free DNA replication fork repair and restart are of critical importance for cancer etiology and therapeutic susceptibilities. Yet, damaged and stalled forks occur frequently and must be both repaired and accurately restarted by nucleases, helicases, and other replication factors. Here, we identified human exonuclease V (hExo5) as the newest and perhaps least understood component of replication fork restart pathway. hExo5 is a 5' to 3' single-strand DNA (ssDNA) exonuclease that does not cut blunt-end double-strand DNA (dsDNA) and circular DNA [1]. Interestingly, higher *EXO5* gene expression correlates with lower survival rate in Adrenocortical carcinoma patients. We showed *EXO5*-knocked down (KD) cells are sensitive to interstrand crosslink DNA damaging agents, cisplatin and camptothecin, and have slower replication restart rate after fork stalled or collapsed. We further identified that *EXO5* colocalizes with RPA and BLM helicase at the damage foci. Moreover, *BLM*-KD and/or *EXO5*-KD cells showed the same level of cell survival after cisplatin treatment indicating they function in the same pathway. To understand how hExo5 functions with DNA mechanistically, we solved the crystal structures with and without DNA substrate (Fig. 1). The structures showed hExo5 contains an [4Fe-4S] cluster domain that discriminates between ssDNA and dsDNA and exhibits structure core similar to PD-(D/E)XK superfamily active site arrangement resembling DNA2 and Cas4 nucleases. Moreover, residue Y221F substitution near the active site abolishes the nuclease activity. hExo5 with ssDNA bound structure reveals Y221 interacts with DNA phosphate group critically positioning DNA substrate for cutting. Interestingly, upon DNA binding, a helix-turn-helix motif moves out of the active site to allow ssDNA threading through the [Fe-S] cluster domain. Together, we propose our integrated model for how hExo5 functions at the replication fork.



### Reference

[1] Sparks *et al.* (2012). *J. Biol. Chem.* **287**, 42773-42783.