Project number 15

Polymerase β and its interacting proteins influencing cellular senescence and cancer

[1]Research group
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[2]Research setup

POLβ plays a key role in base exciion repair (BER) in nucleus and loss of POLβ results in severe BER defects and embryonic lethality in mice. We found in our previous collaboration of this IDAC grant the presence of POLβ in mitochondrial extracts of mouse cell (1). This discovery of POLβ in the mitochondria suggests that it participates in

mitochondrial BER. POLγ has been thought to be the sole polymerase in mitochondria. Therefore, the BER capabilities of POLγ and POLβ were determined if the two enzymes have separate or overlapping functions in the mitochondria or if they cooperate in replication or DNA repair. As a specialized polymerase for DNA repair, POLβ is known to efficiently insert nucleotides in single nucleotide gapped BER intermediates. We found here that POLβ is much more active as a BER polymerase than POLγ on every repair intermediate tested and that mitochondria lacking POLβ are dramatically impaired in BER function (2).

[3]Research outcomes

(3－1)Results

We showed a side-by-side comparison of BER activities of POLβ and POLγ, the mitochondrial replicative polymerase, previously thought to be the only mitochondrial polymerase. We found that POLβ is significantly more proficient at single-nucleotide gap filling, both in substrates with ends that require polymerase processing, and those that do not. We also show that POLβ has a helicase-independent functional interaction with the mitochondrial helicase, TWINKLE. This interaction stimulates strand-displacement synthesis, but not single-nucleotide gap filling. Importantly, we find that purified mitochondrial extracts from cells lacking POLβ are severely deficient in processing BER intermediates, suggesting that mitochondrially localized DNA POLβ may be critical for cells with high energetic demands that produce greater levels of oxidative stress and therefore depend upon efficient BER for mitochondrial health (2).

(3－2)Future perspectives

Here, we have shown the importance of mitochondrial POLβ in the repair replication in BER. Akira Yasui's laboratory has previously shown that the three major human glycosylases for the repair of oxidatively damaged bases functioning in BER, hOGG1, hMYH1 and hNTH1 are targeted to mitochondria as mitochondrial proteins created by alternative splicing of each mRNAs (3, 4). Thus, in addition to the glycosylases, POLβ is a member of here, the major players for BER in the nucleus were identified in the mitochondria as well. Therefore, the defect in POLβ or glycosylases can be influential in both nucleus and mitochondria. The consequence of this finding will be important in cancer and aging caused by BER deficiency.

[4]List of Papers

1. Sykora P, Kanno S, Akbari M, Kulikowicz T, Baptiste BA, Leandro G, Lu H, Tian J, May A, Becker KA, Croteau DL, Wilson DM III, Sobol RW, Yasui A and Bohr VA. DNA polymerase b participates in mitochondrial DNA repair. *Mol*

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2. Baptiste B, Baringer SL, Kulikowicz T, Sommers JA, Croteau DL, Brosh RM Jr, and Bohr VA. DNA polymerase ß outperforms DNA polymerase γ in key mitochondrial base excision repair activities. *DNA Repair* 99, 2021, 103050.

3. Takao M, Aburatani H, Kobayashi K, and Yasui A. Mitochondrial targeting of human DNA glycosylases for repair of oxidative damage. *Nucleic Acids Res.*, **26**, 2917–2922, 1998.

4. Takao M, Zhang QM, Yonei S, and Yasui A. Differential subcellular localization of human homolog (*hMYH*) and the functional activity of adenine:8-oxoguanine DNA glycosylase. *Nucleic Acids Res.* **27**, 3638-3644, 1999.