NIST researchers develop a novel approach for the measurement of a crucial DNA repair enzyme in human cells

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(Phys.org) —NIST researchers recently reported a novel approach for identifying and quantifying an important DNA repair enzyme in mammals including humans. This enzyme, apurinic/apyrimidinic endonuclease 1 (APE1), plays a critical role in base excision repair of DNA damage. APE1 plays a key role in repairing mutagenic base-free sites in DNA by first generating strand breaks which paves the way for other enzymes to fully repair DNA.

It has been shown that mice with a deleted gene coding for APE1 cannot survive, underscoring the critical nature of this enzyme. APE1 also plays an important role in dictating DNA repair capacity, which can determine how patients respond to cancer therapies. High expression of APE1 has been associated with resistance to therapy for many cancers. Mounting evidence points to the predictive and prognostic value of APE1 expression and subcellular localization in cancers. APE1 nuclear localization typically associates with a good prognosis, whereas combined cytoplasmic and nuclear localization correlates with poor survival. Moreover, mutations to APE1 are thought to dispose individuals to cancer.

Therefore, accurate measurements of APE1 levels in human tissues are essential for evaluating APE1 as a predictive and prognostic biomarker in cancer and guiding cancer treatments. Researchers in NIST's Material Measurement Laboratory developed a novel approach for the positive

identification and absolute quantification of APE1 in human cells using liquid chromatography-isotope-dilution tandem mass spectrometry (LC-MS/MS). Researchers first expressed and purified a full-length human isotopically-labeled APE1 from genetically engineered E. coli cells, which was used as an internal standard for MS measurements.

Hydrolysis of APE1 and 15N-labeled APE1 with a proteolytic enzyme and subsequent analysis by LC-MS/MS resulted in identification of numerous peptides. Subsequently, APE1 was positively identified and quantified in nuclear and cytoplasmic extracts of multiple human normal and cancer cell lines. Nuclear extracts showed greater levels of APE1 than cytoplasmic extracts and cancer cells exhibited greater expression level of APE1 than normal cells. The same approach was also used to measure APE1 in mouse tissue, and APE1 variants found in humans. Overall, this work is an important first step for understanding the role of this enzyme in disease and therapeutic efficacy. Future studies will explore the broader utility of this method for measuring APE1 in patient samples.

This work has recently been published in the journal *PloS One*.

More information: Kirkali, G., Jaruga, P., Reddy, P. T., Tona, A., Nelson, B. C., Mengxia Li, M., Wilson III, D. M., Dizdaroglu, M. "Identification and quantification of DNA repair protein apurinic/apyrimidinic endonuclease 1 (APE1) in human cells by liquid chromatography/isotope-dilution tandem mass spectrometry," *PLoS One* 8(7), e69894, 2013.

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