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Role of Endonuclease G in Exogenous DNA Stability in HeLa Cells

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Abstract : Endonuclease G (EndoG) is a well conserved mitochondrio-nuclear nuclease with dual lethal and vital roles in the cell. The aim of our study was to examine whether EndoG exerts its nuclease activity on exogenous DNA substrates such as plasmid DNA (pDNA), considering their importance in gene therapy applications. The effects of EndoG knockdown on pDNA stability and levels of encoded reporter gene expression were evaluated in the cervical carcinoma HeLa cells. Transfection of pDNA vectors encoding short-hairpin RNAs (shRNAs) reduced levels of EndoG mRNA and nuclease activity in HeLa cells. In physiological circumstances, EndoG knockdown did not have an effect on the stability of pDNA or the levels of encoded transgene expression as measured over a four day time-course. However, when endogenous expression of EndoG was induced by an extrinsic stimulus, targeting of EndoG by shRNA improved the perceived stability and transgene expression of pDNA vectors. Therefore, EndoG is not a mediator of exogenous DNA clearance, but in non-physiological circumstances it may non-specifically cleave intracellular DNA regardless of its origin. These findings make it unlikely that targeting of EndoG is a viable strategy for improving the duration and level of transgene expression from non-viral DNA vectors in gene therapy efforts.

Keywords : EndoG, silencing, exogenous DNA stability, HeLa cells
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