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PP24-2 APE/Ref-1 deficiency inhibits cellular transformation and metastatic potential of human melanoma cells

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Abstracts

Apurinic/apyrimidinic endonuclease/redox factor-1 (APE/Ref-1) is a multi-functional protein, involved in DNA base excision repair and redox regulation of nuclear transcription factors. Our previous study revealed abnormal Ref-1 levels in melanoma, associated with drug resistance, and demonstrated its critical role in malignant transformation. In combination with other studies, these results suggest that Ref-1 serves as a potential druggable target for melanoma and potentially other malignancies as well. However, Ref-1 protein contains three distinct domains: nuclear translocation sequence (N-terminal residues:1-36); redox regulation domain (residues 43-93 required) overlapping with DNA repair domain (residues 61-80 and all the C-terminus are essential). To provide more detailed information, especially the critical roles of distinct domains in melanoma progression, we built two Ref-1 deficient constructs: nuclear-localization sequence deleted-Ref-1 (NLD-Ref-1) and redox-regulation deficient Ref-1 with cysteine 65/93 replaced by alanine (RedoxD-Ref-1). In Lu1205, stable expression colonies were selected. Growth curves showed that cells carrying deficient Ref-1 exhibited significant inhibition of proliferation. Ref-1 deficiency also remarkably inhibited melanoma cells survival from anoikis in a forced suspension culture, associated with decreased Bcl-2 levels similar in amount depletion of Ref-1. A matrix-adhesion assay revealed that both NLD-Ref-1 and RedoxD-Ref-1 cells significantly reduced adhesion to fibronectin-coated surfaces, with a much slower migration detected by scratch recovery analysis. In addition, melanoma inhibitory activity (MIA) levels, which are abnormally elevated in melanoma cells compared to melanocytes, were decreased markedly in Ref-1 deficient cells as assessed by RT-PCR. We propose that induction of Ref-1 in melanoma plays a critical role in melanoma progression and that new strategies interfering with Ref-1 should be pursued.