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Expression of NADPH oxidase 1 in melanoma cells and its effect on invasion via induction of matrix metalloproteinase-2

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NADPH oxidase (EC 1.6.3.) is a family of enzymes that catalyze transfer of an electron from NAD(P)H to molecule oxygen to generate superoxide or hydrogen peroxide. It consists of seven members, represented by their catalytic subunits: Nox1, Nox2 (gp91phox), Nox3, Nox4, Nox5, Duox1 and Duox2. Among these members Nox4 was previously demonstrated to play a role in melanoma invasion down-stream of Akt pathway. Expression of Nox1 was not reported in melanoma cells. RT-PCR and Western blot analysis was used to analyze expression of Nox1, Noxo1 and p22phox in normal melanocytes and melanoma cells; over-expression, knockdown or inhibition of Nox1 was achieved in radial growth phase Wm3211 melanoma cells, cell invasion was assayed using Matri-gel coated transwells. Expression of MMP-2 was analyzed by qRT-PCR and western blot in these cells. Promoter reporter plasmid of human MMP-2 is being constructed; promoter activity will be analyzed in cells clones with varied Nox1 expression levels. Nox1 was expressed in normal melanocytes and all melanoma cell lines examined, as in contrast to Nox4 which was not expressed in normal melanocytes but only expressed in a subset of metastatic melanoma cell lines. Nox1 subunits Noxo1 and p22phox were also expressed in normal melanocytes and all melanoma cell lines. Over-expression of GFP-fused Nox1 protein in Wm3211 cells increased cell invasion rate by four to six-fold, while knocking down Nox1 decreased invasion rate by two to three-fold. Inhibiting Nox1 by Diphenyliodonium (DPI) also inhibited invasion rate. We further found that secreted MMP-2 increased in cells over-expressing Nox1, and decreased in cells with Nox1 knockdown. Quantitative RT-PCR analysis demonstrated the regulation of MMP-2 may occur at transcription level as mRNA of MMP-2 increased about 10 to 11 fold in cell clones over-expressing Nox1. Our data

shows that Nox1 is over-expressed in all melanoma cell lines examined; it contributes to enhanced cell invasion by activating MMP-2 at transcriptional level in melanoma cells.