# Phosphoproteome of Melan-a, TM1 and TM5 Cells using Liquid Chromatography off-line MALDI-TOF/TOF-MS.

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Melanoma is a highly aggressive tumor derived by transformation of melanocytes due to changing in skin microenvironment. We are studying TM1 and TM5, two murine melanoma cell lineages obtained from a nontumorigenic melanocyte lineage, melan-a, that was submitted to sequential cycles of forced anchorage impediment mimicking melanoma progression (Oba-Shinjo et al. Neoplasia, 8:231-241, 2006). Development of cancer is due to accumulation of gene mutations that affect signaling networks that control cell proliferation and migration. Protein phosphorylation is the central protein modification implicated in control of cell cycle and signal transduction. We report preliminary results of phosphoproteome of melan-a, TM1 and TM5. Phosphoprotein profiling was obtained by western blotting probed with anti-phosphotyrosine and anti-phosphoserine antibodies after SDS-PAGE. We use combination of protein de-phosphorylation and IMAC enrichment of phosphopeptides after *in situ* or solution trypsin digestion. Shotgun proteomics was used for identification of proteins using capillary chromatography with online fraction collection directly on MALDI target and off-line MALDI-TOF/TOF-MS. Two hundred two (202) phosphoproteins were characterized by the presence of one or more phosphopetide sites in melan-a, TM1 and TM5. De-phosphorylation by alkalin phosphatase was not effective and was possible to detect only 11 phosphopeptide sites. Characterization of post-translational modification of proteins like phosphorylation is one of the most challenging tasks due to its highly dynamic nature and low stoichiometry. Supported by FAPESP, FAEPA and FINEP.