Toward quantitative understanding of compartmentalized NADPH metabolism in cancer cells

by

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Technical Summary

Reduced nicotinamide adenine dinucleotide phosphate (NADPH) is an essential molecule in living organisms by serving as an electron donor to drive reductive biosynthesis and protect cells against oxidative stress. Over the past decades, extensive studies have focused on defining key NADPH regeneration pathways and whether targeting these pathways can be effective for cancer therapy. However, fundamental questions still remain unanswered. It is relatively unknown as to the cytosolic and mitochondrial NADPH pool sizes and their dynamics under varying metabolic processes. Assessment of compartmentalized NADPH redox states is important as cytosolic and mitochondrial NADPH levels are known to be different due to its impermeability to inner mitochondrial membrane. Different pool sizes can influence metabolic processes to a varying extent and targeting compartment-specific NADPH dependent enzymes may result in selective responses. Thus, improved understanding of compartmentalized NADPH pool sizes, dynamics and metabolic pathway activities may lead to a designing of effective cancer therapies that target NADPH metabolism.

In this thesis, we investigated compartmentalized NADPH metabolism in cancer cells, using genetically encoded NADPH biosensors, ^{13}C-glcucose isotopic tracers, and mathematical models. First, using NADPH sensors, we observed mitochondrial NADPH pool decreased in response to mitochondria-specific oxidative stress, whereas the cytosolic NADPH was minimally influenced. Second, based on a kinetic model for a mitochondrial antioxidant network, we estimated the extent of mitochondrial NADPH/NADP$^+$ and an activation of indirect NADPH shuttle system was necessary to maintain the mitochondrial NADPH pool upon mito-stress. Third, we found the activity of oxidative pentose phosphate pathway and glucose anaplerosis elevated in response to the decrease of mitochondrial NADPH. Fourth, a genome-scale metabolic model simulation revealed a citrate transporter played a key role for NADPH homeostasis and we observed an inhibition of the transporter decreased the cytosolic NADPH pool. Lastly, we determined the cytosolic and mitochondrial NADPH dynamics varied among different cancer cell lines, and perturbing compartment-specific NADPH pools led to cell line specific growth inhibitions based on the contribution factor analysis *in vitro*. Altogether, our integrated approach and findings provide an insight into compartmentalized NADPH metabolism that may be considered in designing selective anticancer therapies.

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