Kinetic and Characterization of Malic enzyme in human breast cancer and normal tissue

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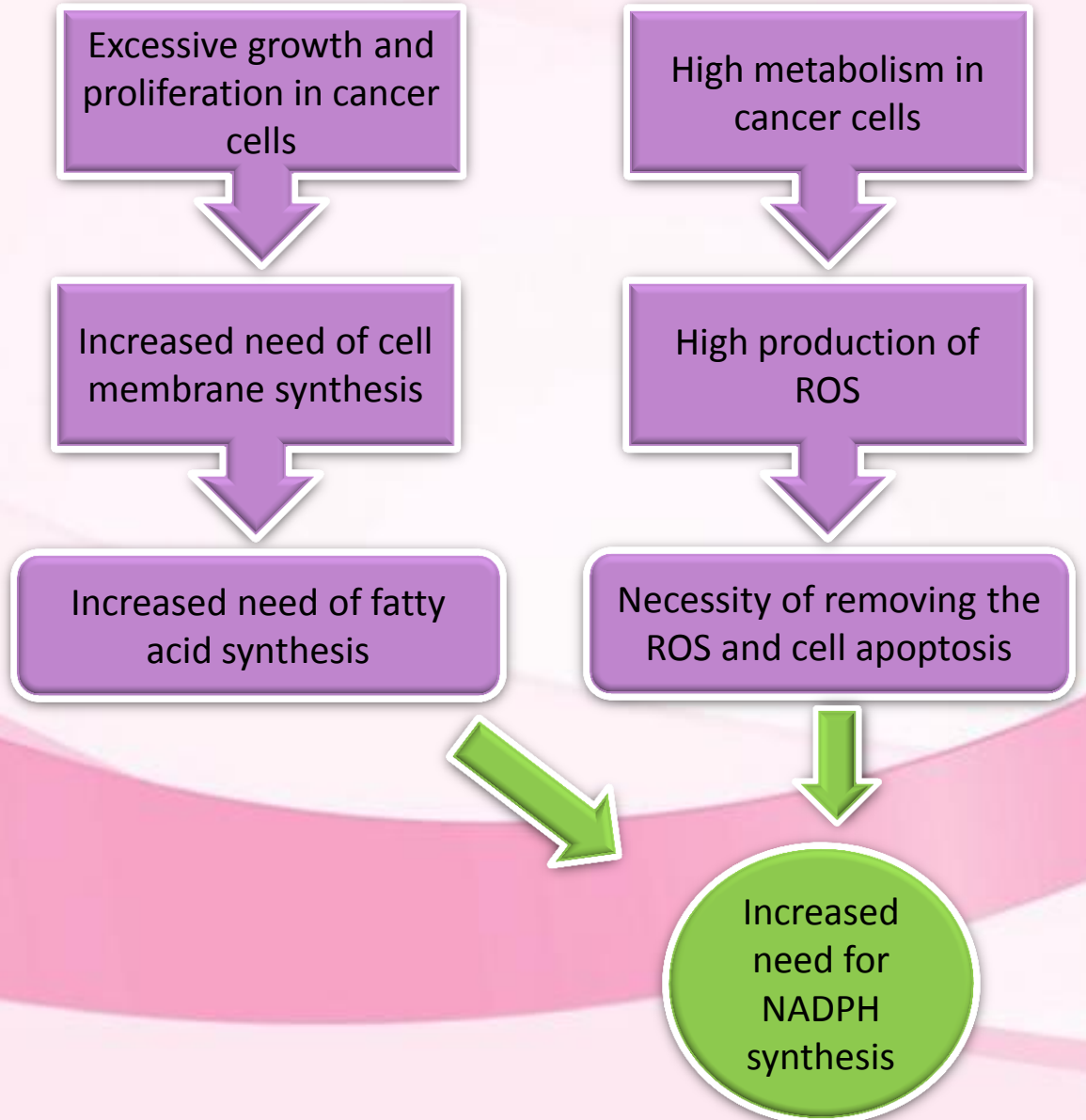
Introduction

Excessive growth in cancer cells

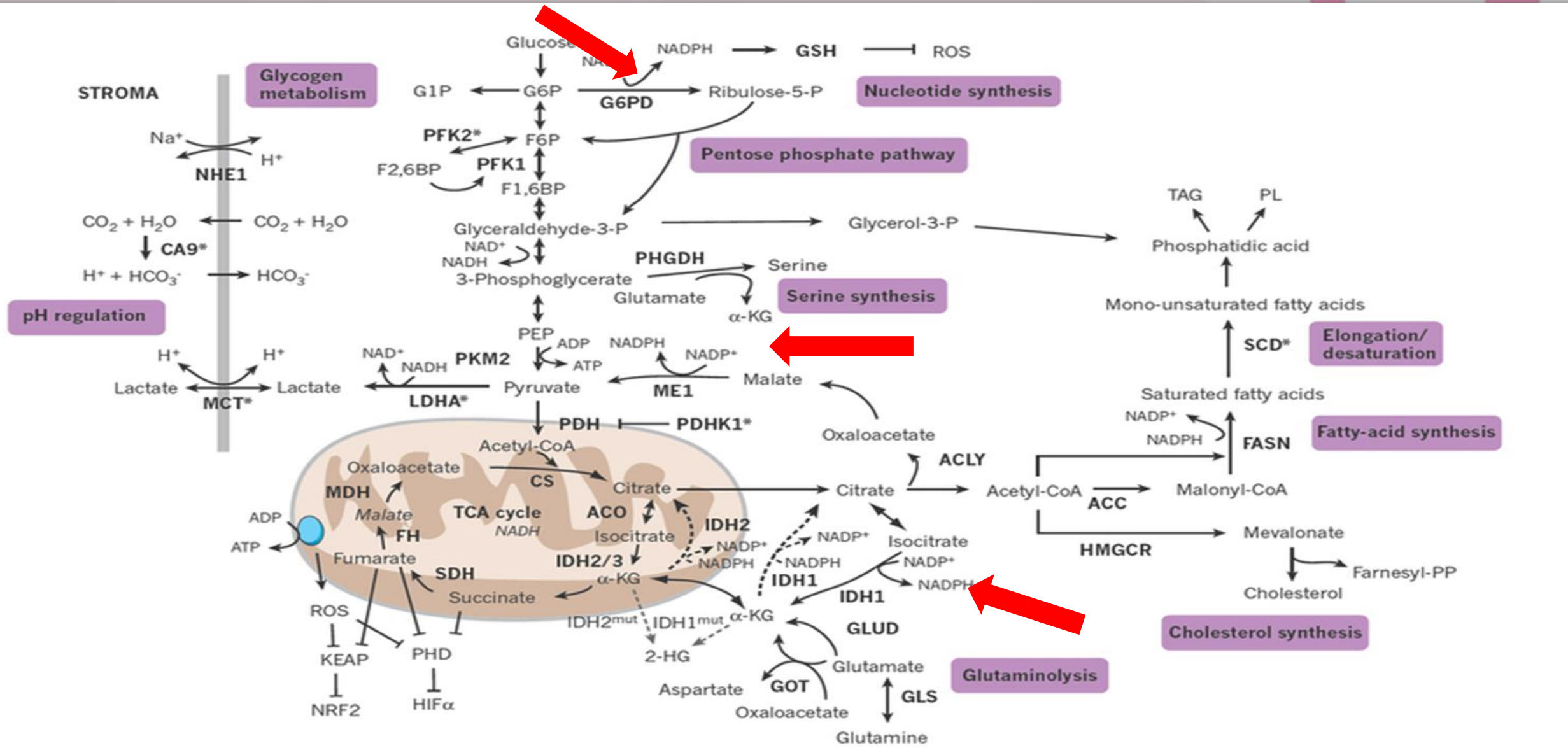
- Excessive growth and proliferation important features of cancer cells.
- cancer cells need energy and cellular components to proliferate.
- high aerobic glycolysis and substantial fatty acid synthesis are the most well-known characteristics of cancer cells metabolism*.
- Some essential metabolites; pyruvate, NAD and NADPH

* Hanahan D, Weinberg RA: Hallmarks of Cancer: The Next Generation. Cell 2011, 144:646-674.

Role of NADPH in cancer cells



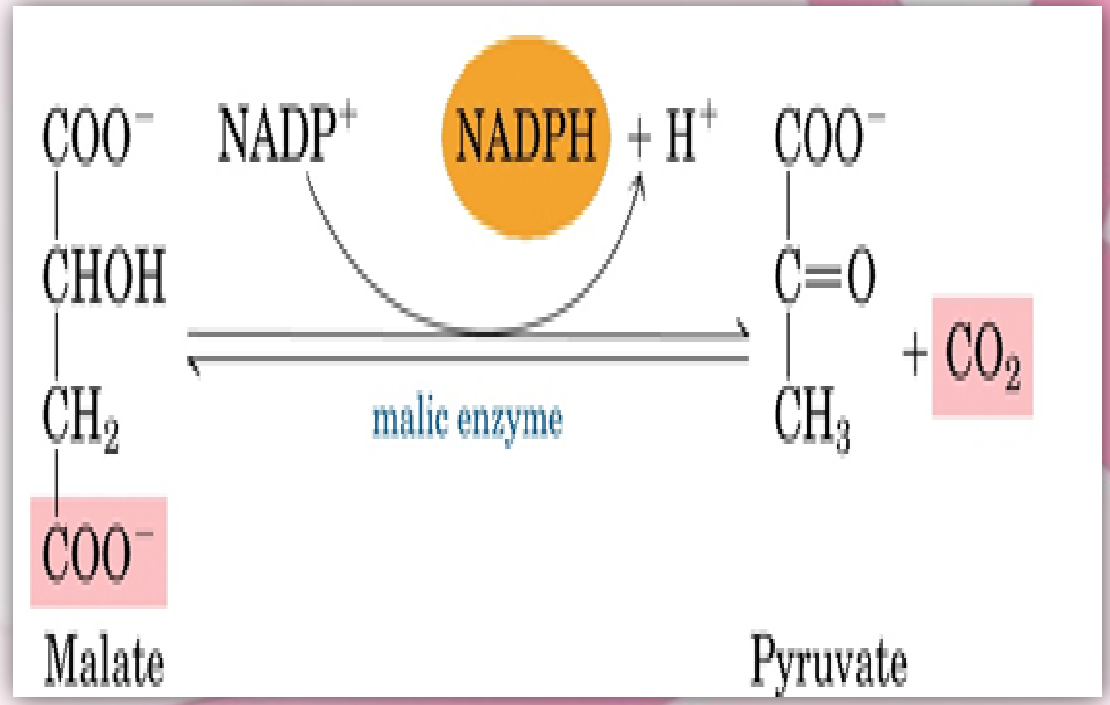
NADPH GENERATING ENZYMES



Malic enzyme

Isoforms of Malic enzyme:

- Cytosolic NADP-dependent malic enzyme
- Mitochondrial NAD(P)-dependent malic enzyme
- Mitochondrial NADP-dependent malic enzyme

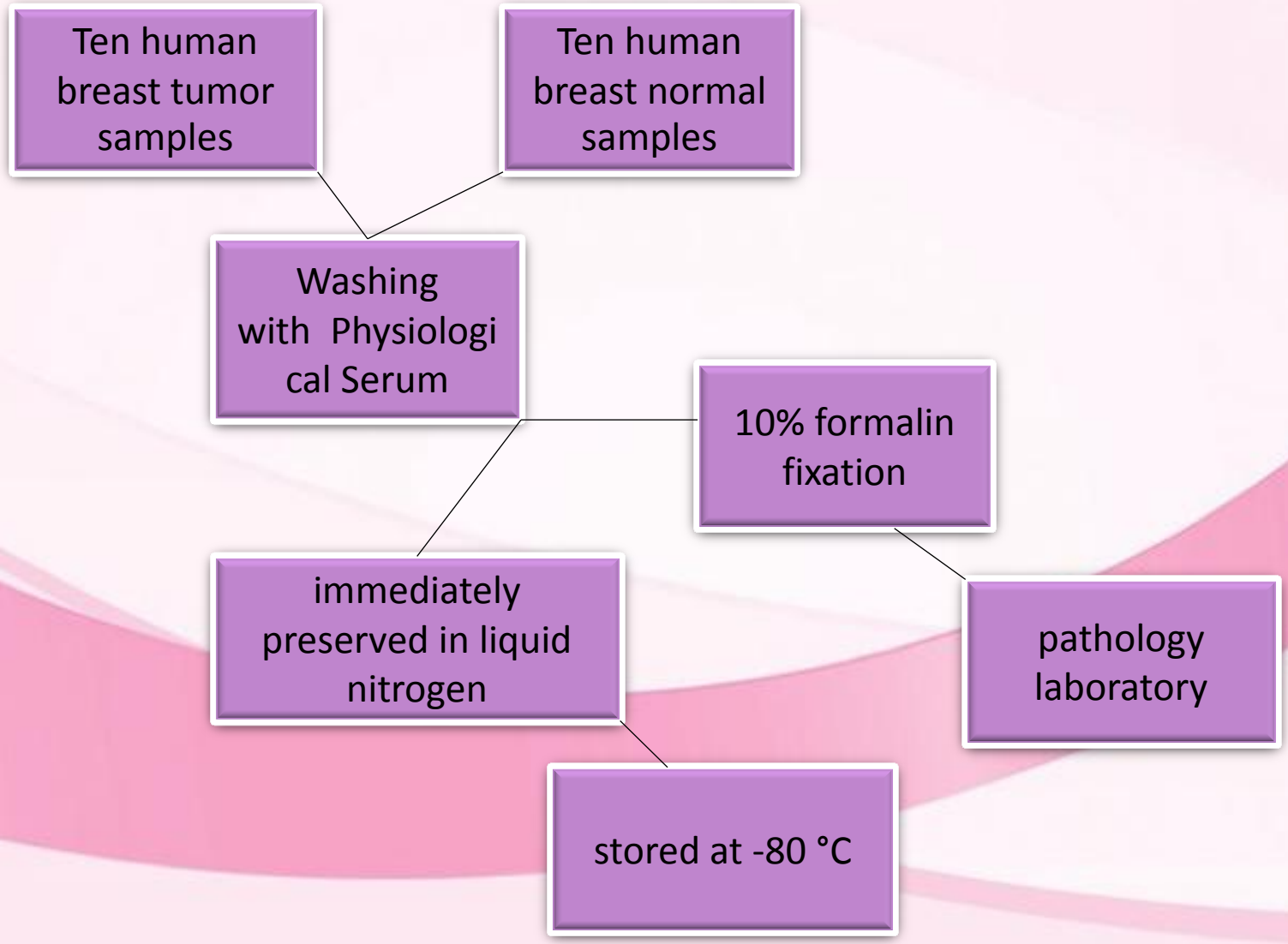


Aim of the study

- The aim of current study was to elucidate kinetic parameters of ME in human breast cancer samples by considering two points of view; the important role of ME in cancer metabolism and the strong effect of microenvironment on enzyme kinetic in which enzyme operates with attention the different characteristics of tumor milieu.

Material and methods

Clinical sample collection



Sample Preparation and ME partial purification

- Frozen were homogenized (1:5 w:v) in ice cold homogenization buffer
- homogenization buffer (20 mMTris–HCl, pH 7.5, 10 mM 2-mercaptoethanol, 10% v:v glycerol, 1 mM EDTA, 1 mM EGTA, and 10 mM β-2ME)
- centrifuged for 30 min at 15000 g at 4 °C and the supernatant was decanted and held on ice until use
- Sephadex G25 columns equilibrated in homogenizing buffer.
- ME partial purification has done with a DEAE-Sephadex column (1.5×10cm)*
- Top activity fractions were then pooled and held out at 4 °C until use..

* Gu-Gang CHANG, Jehng-Kang WANG, Ter-Mei HUANG, Hwei-Jen LEE, Wei-Yuan CHOU and Ching-Liang MENG. Purification and characterization of the cytosolic NADP-dependent malic enzyme from human breast cancer cell lines. Eur. J. Biochem. 202, 681-688 (1991).

Enzyme assay and kinetic parameters

- ME activity was measured in the presence of malate with NADP as substrates and $Mgcl_2$ as cofactor.
- The lowest concentration of each substrate
- Reactions were initiated by adding 25µl of purified enzyme to a 200 µl total reaction volume by using 20mM Tris-Hcl buffer pH 7.5, in the microplate well.
- Activity was monitored at 340 nm for checking the conversion of NADP to NADPH by using a Biotech Powerwave microplate reader and Gen5 software version 2.0 (USA) (kinetic mode, reading interval = 39 s).*

* Brooks SPJA: Simple computer program with statistical tests for the analysis of enzyme kinetics.Biotechniques 1992, 13:906–911.
* Brooks SPJA: Program for analyzing enzyme rate data obtained from a microplate reader. Biotechniques 1994, 17:1155–1161.

Enzyme assay and kinetic parameters

- The Km malate was determined at 1mM NADP and malate concentrations ranging from 4 mM to 126mM.
- The Km NADP was determined at 50mM malate and NADP concentrations ranging from 0.0025 to 0.05mM in both tissues
- Guanidine hydrochloride (GnHCl) and NaCl I_{50}s were performed by assaying tumor and normal partial purified ME at under optimal conditions for the malate-decarboxylation reaction with the addition of various concentrations of the above effectors.

Statistical analysis

- Data were expressed as mean ± SEM from independent determinations on separate preparations of enzyme. Data were analyzed using Student's t-test. The level of significance for all tests was set at $p < 0.05$.

Results

Optimization of experimental conditions

- Optimum assay conditions for ME were 50mM malate and 1mM NADP in both tumors and normal tissues.

ME Partial Purification

- A typical purification experiment

Purification Step	Total Protein (mg)	Total Activity (U)	Specific Activity (U/mg protein)	Fold Purification	% Yield
Supernatant	8.5	0.027	0.0031	-	-
DEAE-Sephadex	1.9	0.0155	0.0081	2.61	55

Purification scheme for ME in normal breast samples

Kinetic properties of ME

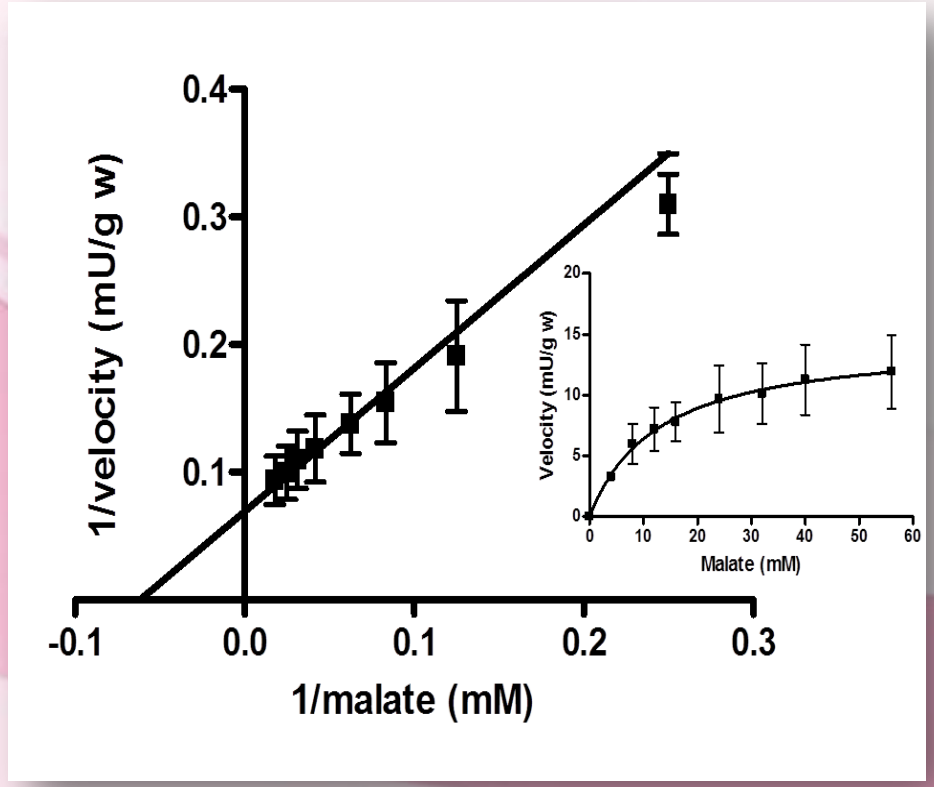
	Tumor	Normal
$K_m$malate (mM)	6.02± 1.6*	12.78±4.73
K_mNADP (mM)	0.022±0.004	0.0175±0.0054
V_{max}malate (mU/gwet weight)	35.21±2.29*	14.61±2.7
V_{max} NADP (mU/gwet weight)	42.68 ±0.51*	34.11±5.61

Kinetic parameters of ME in from breast tumors) and normal tissues

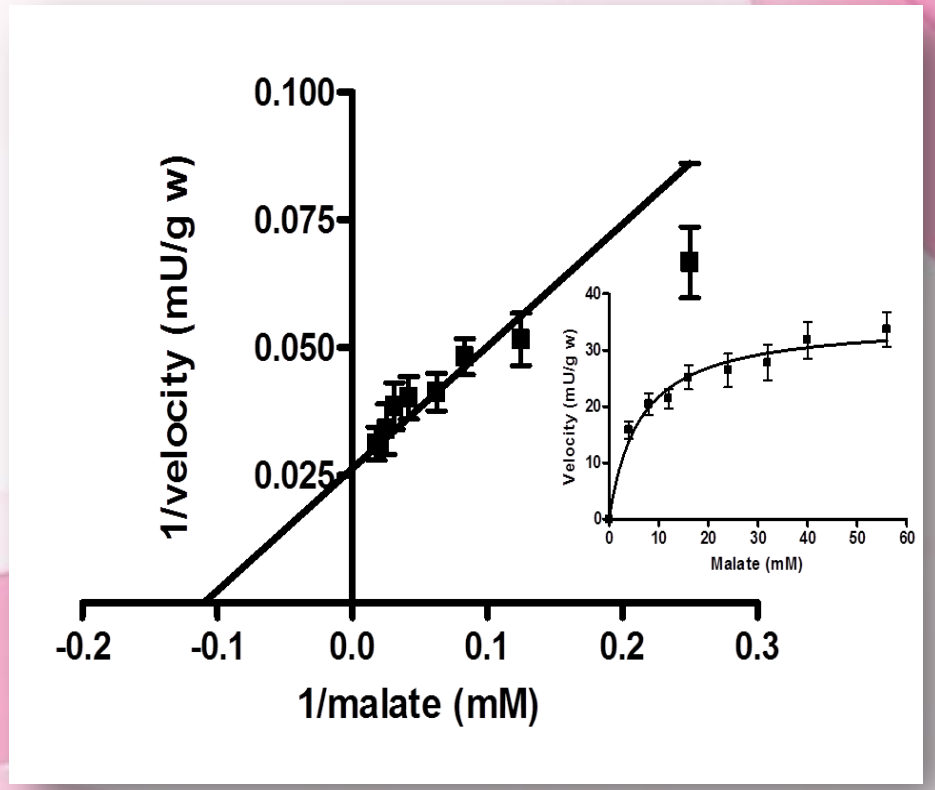
Assays were conducted at 25°C and data are presented as means ± SEM, n=3 independent determinations on each of tumor and normal samples.*Significant difference in each row at $p < 0.05$.

- The $K_m$malate of N-ME (12.78±4.73mM) was significantly higher than that of C-ME (6.02±1.6mM) ($p<0.05$) but there is not any meaningful difference between the K_m $NADP^+$ in normal tissues (0.0175+0.0054mM) and tumor tissues(0.022+0.004mM)

malate control



malate tumor



Structural characteristics of ME

The effects of salt, common denaturant on breast tumors and normal tissues ME

	Tumor	Normal
NaCl I_{50} (M)	1.45±0.25*	1.32±-0.17
GnHCl I_{50} (M)	0.19±-0.03*	0.16±-0.01

Assays were conducted at 25°C and data are presented as means ± SEM, n=3 independent determinations on each of tumor and normal samples. * Significant difference in each row at $p < 0.05$.

Discussion

Discussion

- Tumor microenvironment has distinct feature in compared to normal tissue.
- tumor microenvironment a stressful milieu in which cells have to adapt to these conditions in order to growth and proliferate.
- Enzyme function is one of the most effectiveness approaching order to construct new metabolic capacities to adapt to stressful milieu.*

* Keneth B. Storey and Stephen P.J. Brooks.Principles of Medical Biology, Volume 4. Cell Chemistry and Physiology: part 1, pages 147-169. 1995 by JAI Press Inc.

Our data showed ...

- The tumor ME has lower km of malate and also higher maximum velocity in compared to normal tissues.
- The maximum activity and kinetic we measured was due primarily to the cytosolic fraction
- The enzyme partial purification by DEAE was free of LDH and MDH
- Vmax of ME in tumors was higher than normal tissues.

Advantages of Lower km of malate and higher maximum velocity of ME for tumors:

- This feature of tumor ME has some advantages for cancer cells:

1. The ME by converting malate to pyruvate produces abundance pyruvate that is subsequently changed to lactate by LDH, this reaction is prominent in cancer cells
2. The ME produces NADPH by catalyzing the reaction of malate to pyruvate. NADPH was yielded by this reaction can be used in some different ways that are very vital and important for cancer cells

ME and fatty acid synthesis

- uncontrolled proliferation rate to produce daughter cells,
- each cell to proliferate needs cellular components such as cell membrane that fatty acid is the most important part of it.
- Tumor ME by more level NADPH production could supply required NADPH for fatty acid synthesis.
- Abundant NADPH production also causes the viability of cells by maintaining caspase2 in an inhibitory state thus an anti apoptotic effect*

*Marisa R. Buchakjian and Sally Kornbluth.The engine driving the ship: metabolic steering of cell proliferation and death. Nature 2010,11,715-727.
nutt,L et al. Metabolic regulation of oocyte cell death through the CaMKII-mediated phosphorylation of caspase-2. Cell 2005,123;89-103.

extra role of NADPH in cancer cells

- Additional NADPH forming metabolic pathways; Pantose Phosphate Pathways (PPP) and isocitrate dehydrogenase*.
- It is unlikely that cytosolic ME contribute significantly to NADPH pool in cytoplasm of tumor cells.
- Extra role of NADPH in cancer cells except to the metabolism.
- NADPH has a key role in anti oxidant system as a cofactor.

* Francesco Belflore, Vito Borzi, Luigi Lo Vecchio, Elena Napoli, and Agata M. Rabuazzo.Enzyme Activities of NADPH-Forming Metabolic Pathways in Normal and Leukemic Leukocytes. Clinical Chemistry 1975,21/7;880-883

Structural studies

- GnHCl as a denaturant → The experiment indicated that the normal ME was more susceptible than tumor ME.
- common salt, NaCl → The assay indicated that tumor ME was slightly less susceptible to NaCl inhibition as compared to normal ME

Thank You!

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