ALTERED ANTIOXIDANT PROFILE AND CARBOHYDRATE METABOLISM IN CANINE MAMMARY TUMORS

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INTRODUCTION

- Mammary tumors are the most common and prevalent type of neoplasms in canine species.

- ROS can cause DNA damage, activate pro-carcinogens, and alter the cellular antioxidant defense system (Ray and Husain,1990).

- Altered antioxidant enzymes during carcinogenesis (Yi sun 1990, Kumaramurugappan *et al*.,2013)

INTRODUCTION

- high rate of proliferation
- High ATP
- Increased biosynthesis of macromolecules

- Tightened maintenance of appropriate cellular redox status.

- High rate of glucose metabolism is metabolic hallmark of rapidly dividing cells (De Berardinis *et al*., 2008).

INTRODUCTION

- High rate of cellular proliferation in hypoxic tumor environment hinders the aerobic pathways inside the cell.
- Cell metabolism is shifted towards glycolysis.
- Lack of data about metabolic alterations in canine mammary tumors.

MATERIALS AND METHODS

Parametre	Method
TBARS	Okhawa et al., 1975
GSH	Ellman, 1959
GPx	Rotruck et al.,1973
GST	Habig, 1974
Catalase	Misra & Fridovich, 1972
Protein	Lowry et al., 1951
Hexose	Niebes, 1972
Hexokinase	Brandstrup et al., 1957
Glucose-6-phosphatase	Koida and Oda, 1959
Fructose-1,6-bisphosphatase	Gancedo and Gancedo, 1971
Glucose-6-Phosphate Dehydrogenase	Ellis and Kirkman, 1961

RESULTS

Parameter	Normal mammary gland	Mammary tumour
TBARS (µmoles/g tissue)	13.73±1.37[a]	49.17±3.81[b]
Glutathione (mg /100g tissue)	32.0 ±1.34[a]	134 ±9.6[b]
Catalase (U/mg protein)	0.71 ±0.04[a]	0.26 ±0.003[b]
GPx (U/mg protein)	7.07 ±0.7[a]	1.27 ±0.04[b]
GST (U/mg protein)	10.58 ±0.65[a]	2.64 ±0.26[b]

Values are Mean±SE

Means are significantly different ($p < 0.001$)

Parameter	Normal mammary gland	Mammary tumour
Hexose (mg/g tissue)	184 ±15.54[a]	506.67 ±28.0[b]
Hexokinase (µ moles of glucose phosphorylated/min/mg protein)	0.41 ±0.02[a]	0.66 ±0.03[b]
Glucose-6-phosphatase(µ moles of inorganic ph released/min/mg protein)	0.067 ±0.0041[a]	0.045 ±0.0013[b]
Fructose-1,6-bisphosphatase (µ moles of inorganic ph released/min/mg protein)	7.04 ±0.15[a]	3.96 ±0.15[b]
Glucose-6-phosphate dehydrogenase(mIU/mg protein)	0.245 ±0.011[a]	0.054 ±0.002[b]

Values are Mean±SE

Means are significantly different ($p < 0.001$)

DISCUSSION

- Increase in lipid peroxidation may be due to the overproduction of oxygen free radicals.
- Damage to biomolecules such as DNA, lipids, proteins and carbohydrates (Datta *et al*., 2000).
- MDA reacts with DNA to form adducts and induces mutations in proto-oncogenes and tumor suppressor genes which lead to transformation (Burcham, 1998).
- Further, rapidly dividing cells causes excess production of oxygen free radicals due to increased metabolism (Kumaramurugappan *et al*., 2005).

GLUTATHIONE

- Glutathione, a thiol containing compound.
- Elevation of GSH levels is an early proliferative response (Poot *et al*., 1995).
- Maintain the reduced status of thioredoxin which is essential for DNA synthesis (Lu, 1999).
- The reduced activities of GPx, GST and catalase in cancer tissues are responsible for increase in TBARS formation.

  This may be due to the excessive utilization of enzymes in conjugation process.

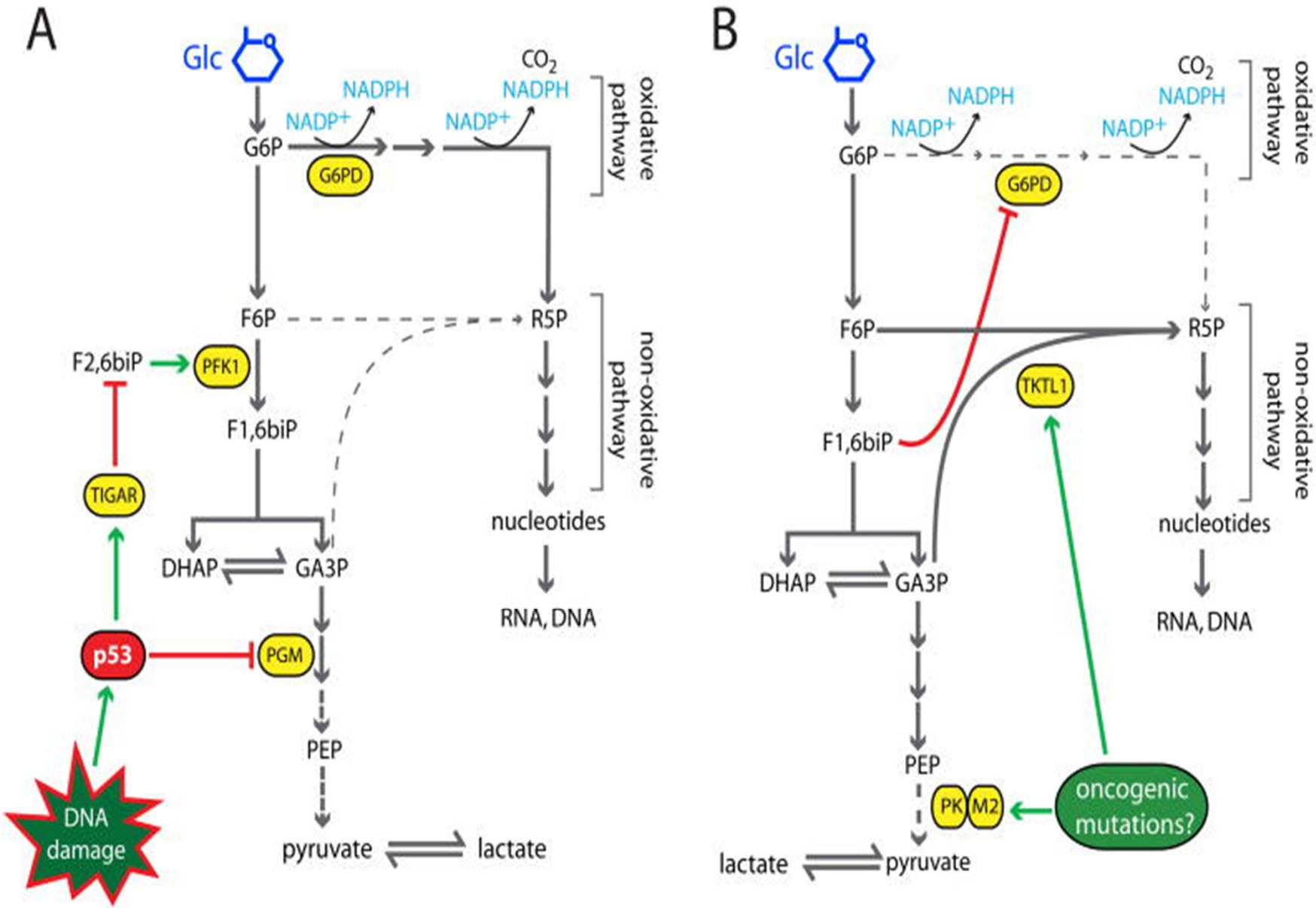
- Normal cells proliferation resulting from growth factor stimulation requires ROS signaling.
- The constitutive activation of this pathway in cancer cells is associated with basal increase in oxidant signaling.
- Neoplastic transformation is associated with an increase in the basal level of ROS mediated signaling (Schumaker, 2006).
- Chronic increases in ROS may trigger transformation and contribute to cancer progression by amplifying genomic instability.

- The warburg effect of aerobic glycolysis is a key metabolic hallmark of cancer (Hsu and Sabatini, 2008).
- The increased hexose content in the tumor tissues.
- Smith, 2000 - increase in hexokinase activity in breast tumors cases.
- Glucokinase and Hexokinase 2 have the ability to suppress apoptosis through the interaction with mitochondria and suppression of Cytochrome C release (Bryson JM *et al*., 2002).

- Glucose-6-phosphatase is a marker enzyme for liver microsomal activity and it is greatly inhibited in cancer bearing animals.

- Langeswaram *et al*., 2013 observed decreased levels of G-6-phosphatase and F-1, 6-bisphosphatase in liver of patients with breast cancer.

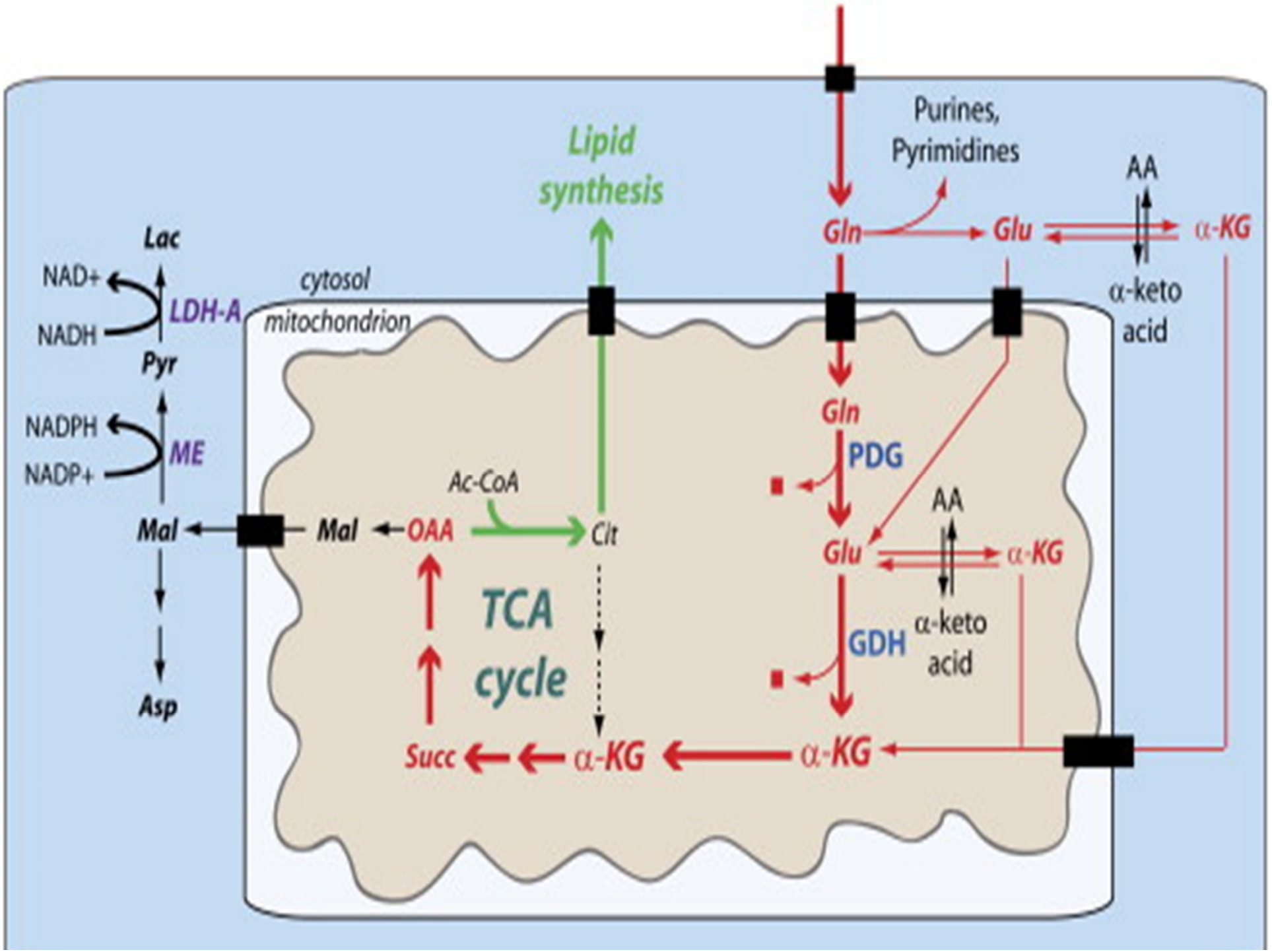
- This may be due to the flux of intermediates of glycolysis to oxidative and non-oxidative phase of pentose phosphate pathway (De Berardinis *et al*., 2008).


A
Glc
NADPH
NADP+
G6P
G6PD
CO2
NADPH
NADP+
oxidative pathway
F6P
R5P
non-oxidative pathway
F2,6biP
PFK1
F1,6biP
TIGAR
nucleotides
DHAP
GA3P
RNA, DNA
p53
PGM
PEP
DNA damage
pyruvate
lactate
B
Glc
NADPH
NADP+
G6P
G6PD
CO2
NADPH
NADP+
oxidative pathway
F6P
R5P
TKTL1
non-oxidative pathway
F1,6biP
nucleotides
DHAP
GA3P
RNA, DNA
PEP
PK
M2
oncogenic mutations?
lactate
pyruvate

- In cancer tissue the HMP pathway also operates at a high rate.

- The oxidative phase of HMP pathway provides the NADPH while the non oxidative phase gives the Ribose-5-Phosphate.

- The decreased G-6-P dehydrogenase activity in the cancer tissues might suggest a state in which the cells need for Ribose-5- Phosphate outweighs its need for glucose derived NADPH (De Berardinis *et al*., 2007).


Lipid
synthesis
Purines,
Pyrimidines
Gln
Glu
AA
α-KG
α-keto
acid
Lac
NAD+
NADH
LDH-A
Pyr
cytosol
mitochondrion
NADPH
NADP+
ME
Mal
Asp
Ac-CoA
Mal
OAA
Cit
TCA
cycle
Succ
α-KG
Gln
PDG
Glu
AA
α-KG
α-keto
acid
GDH
α-KG

- Tumor cells engaged in this form of metabolism have G-6-P dehydrogenase independent NADPH supply.i.e glutamine dependent pathway .

- There is also evidence that some glutamine derived carbon can exit TCA cycle as malate and serve as substrate for maleic enzyme which produces NADPH (De Berardinis *et al*., 2008).

- Glutamate can be directly converted into GSH by the enzyme glutathione cysteine ligase (Cairns *et al*., 2011).



CONCLUSION

- We conclude that the high basal level of oxidative stress with altered enzyme activities of carbohydrate metabolism and G6PD independent supply of reducing equivalents was observed in canine mammary tumor.

- However, much more clinical data will be required in order to discern definite enzyme patterns.

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Thank you...