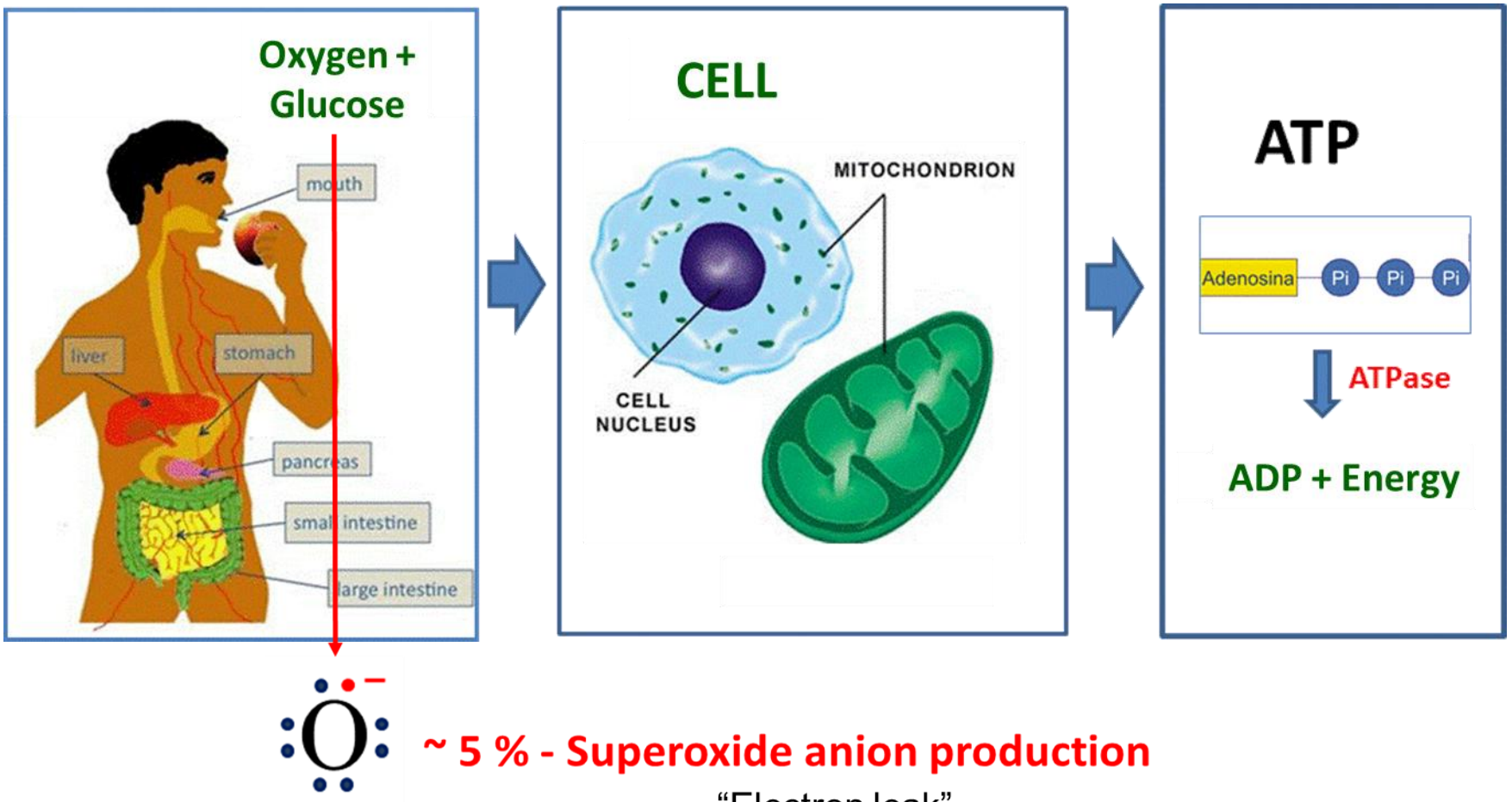
The impact of superoxide-hydrogen peroxide imbalance on cellular dysfunction, senescence and on regenerative therapies

Ivana Beatrice Mânica da Cruz, MSc, PhD
Associate Professor
Universidade Federal de Santa Maria, RS - Brazil

ENERGY PRODUCTION: AEROBIC ORGANISMS

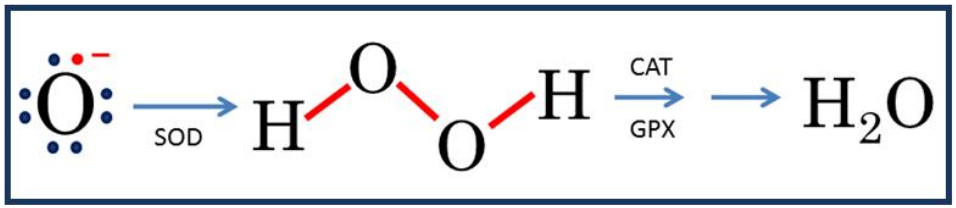


~ 5 % - Superoxide anion production

"Electron leak"

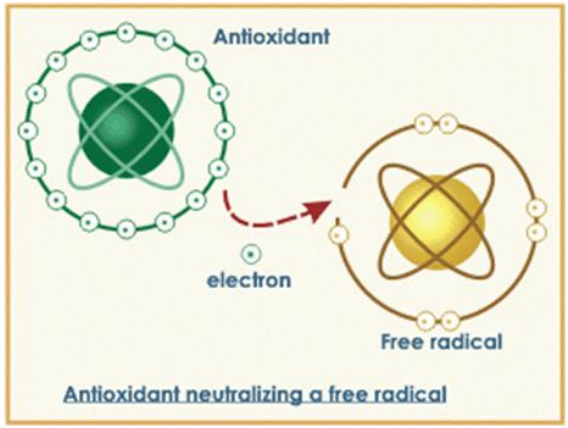
ANTIOXIDANT SYSTEMS

ENDOGENOUS →

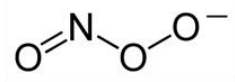


EXOGENOUS →



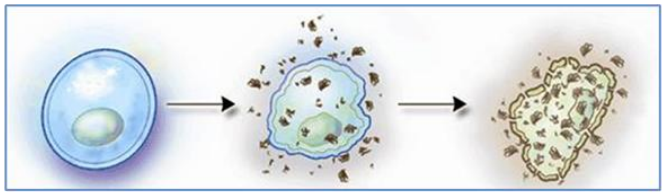


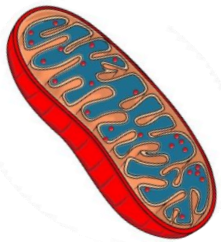
CONSEQUENCES OF SUPEROXIDE AND HYDROGEN PEROXIDE IMBALANCE



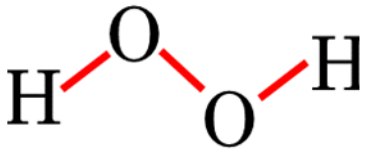
NITRIC OXIDE → PEROXINITRITE → LIPOPEROXIDATION

+





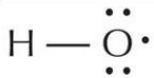




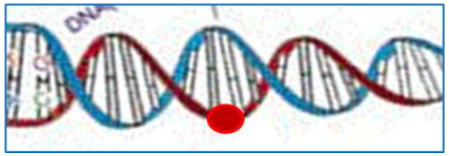
H_2O

Fenton Reaction

+



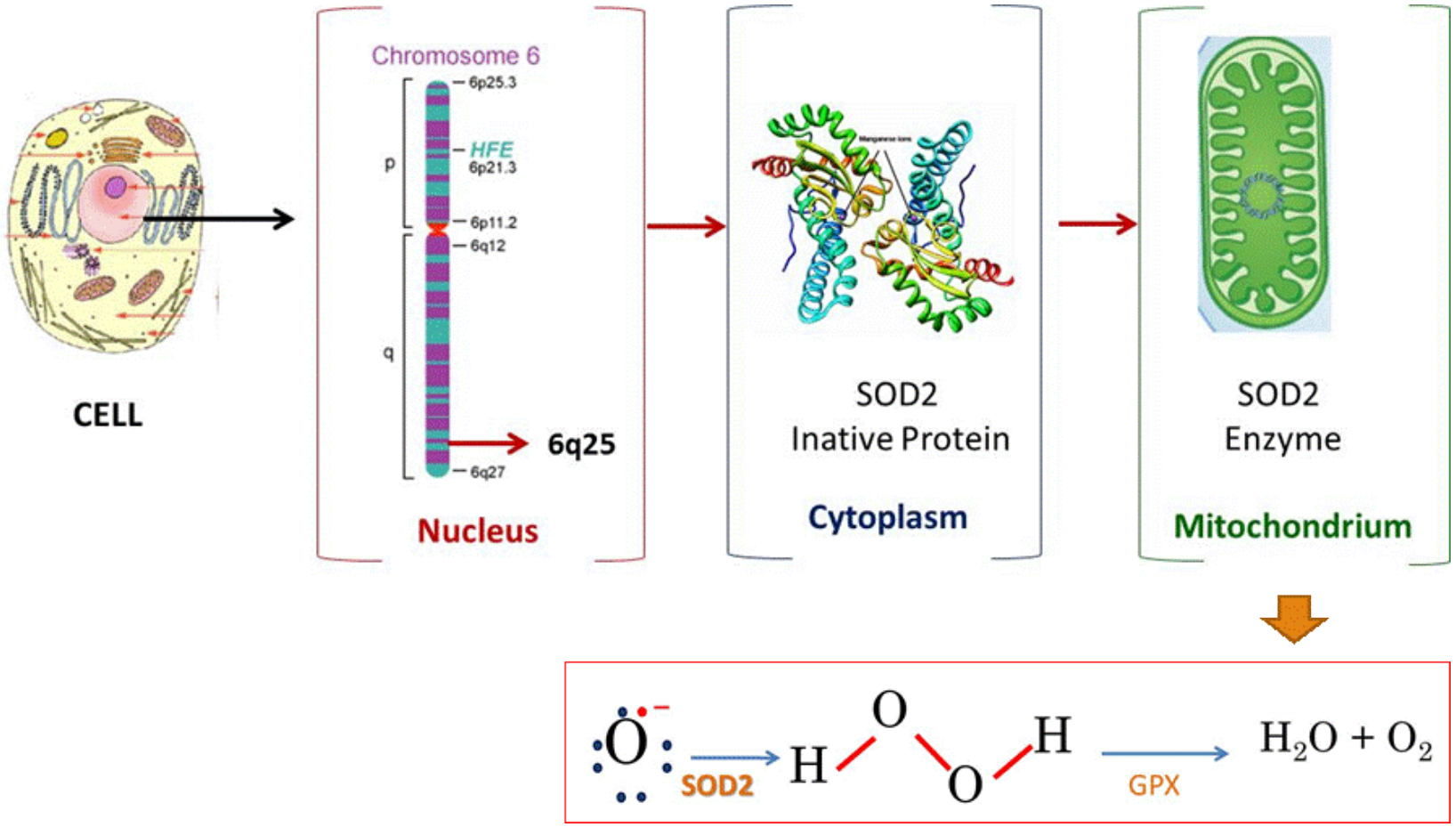
Fe/Zn → OH- → DNA DAMAGE



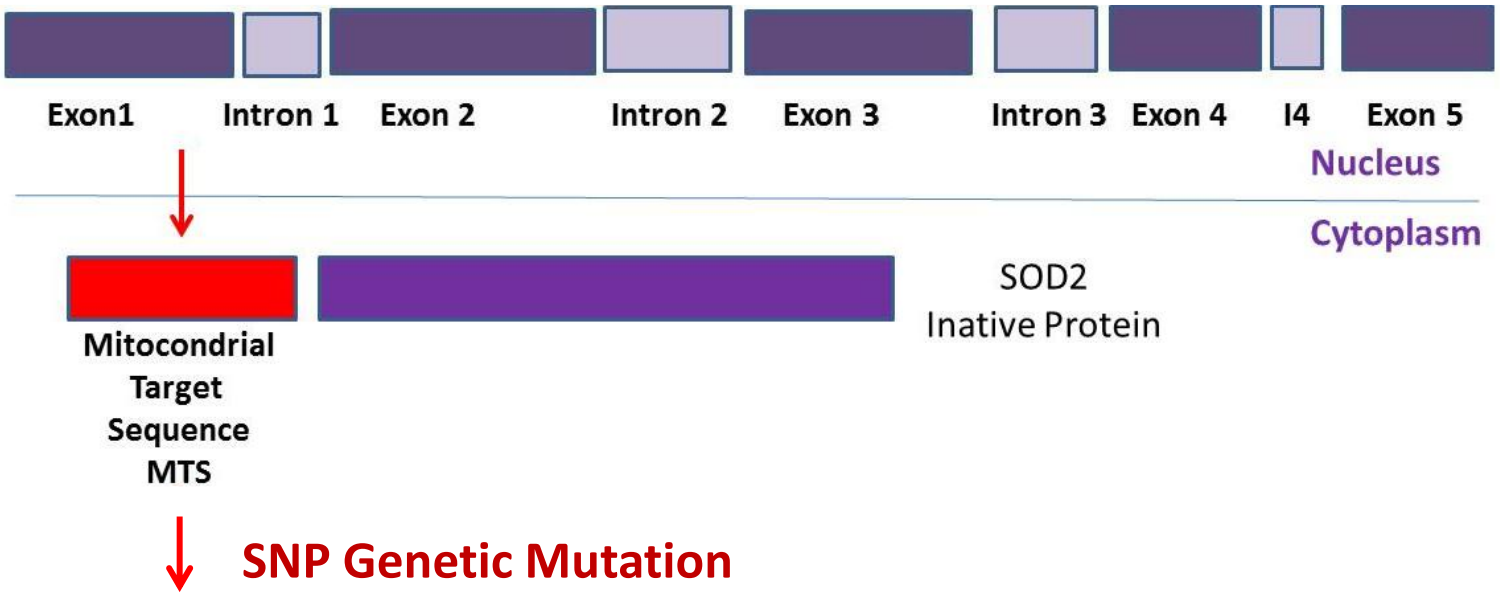
Previous epidemiological results from Ala16Val-SOD2 gene polymorphism

Genetic variaton in Superoxide Dismutase Manganese Dependent an antioxidant enzyme that works into Mitochondria

ALA16VAL-SOD2 GENE POLYMORPHISM

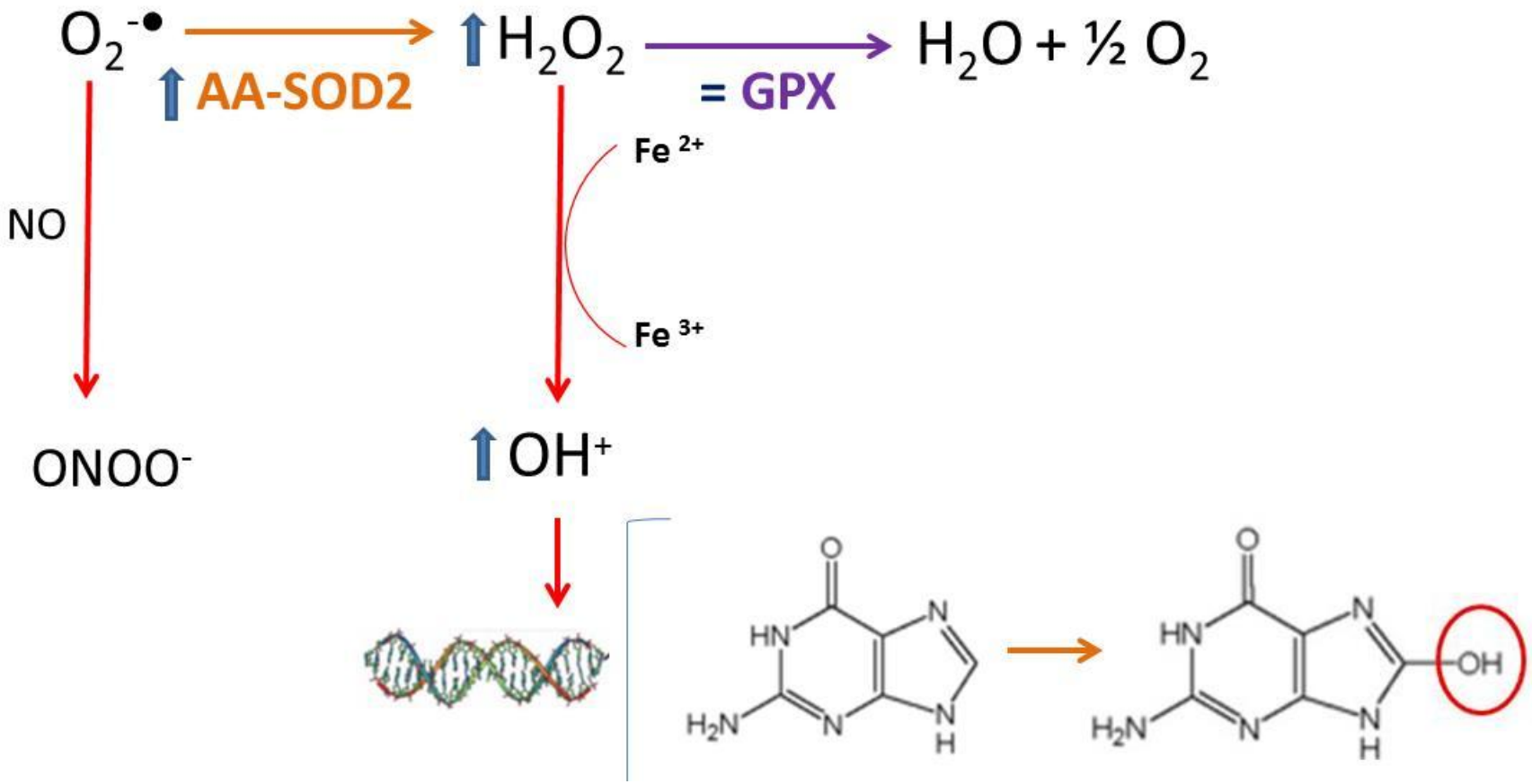


ALA16VAL-SOD2 GENE POLYMORPHISM

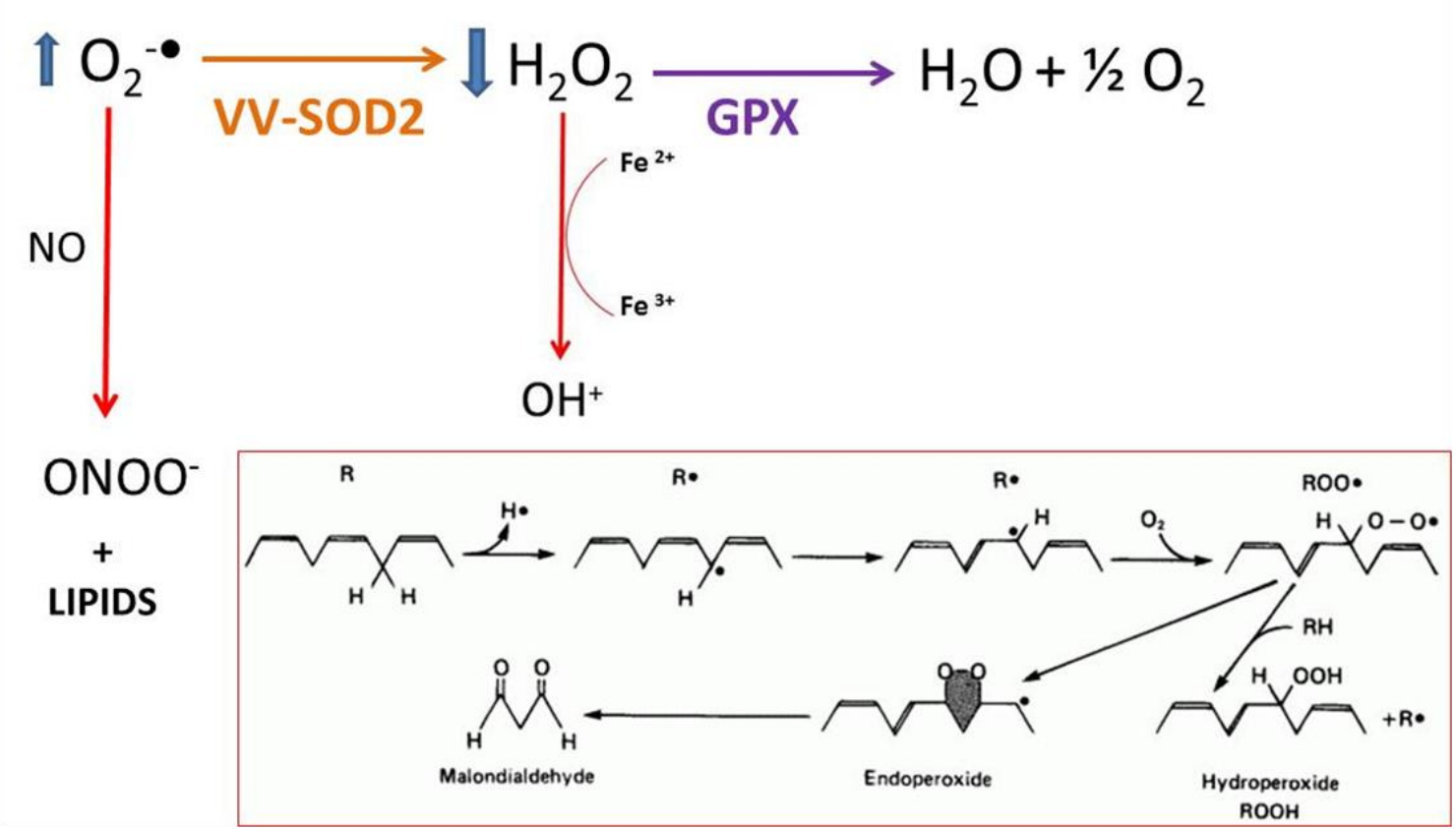


Alleles	Amino acids	Protein Structure	Enzymatic efficiency	Genotypes
Citosine (C)	Alanine	Alpha helix	40% higher	AA
			Intermediary	AV
Timine (T)	Valine	Beta-sheet	Lower	AV

AA GENOTYPE


$O_2^{-\bullet}$
AA-SOD2
H_2O_2
= GPX
$H_2O + ½ O_2$
NO
Fe^{2+}
Fe^{3+}
$ONOO^-$
OH^+
HN
H_2N
N
N
H
O
OH

VV GENOTYPE


↑ $O_2^{-\bullet}$
VV-SOD2
↓ H_2O_2
GPX
$H_2O + ½ O_2$
NO
Fe^{2+}
Fe^{3+}
OH^+
$ONOO^-$
+
LIPIDS
R
R•
R•
ROO•
H•
H
H H
H
O_2
H O – O•
RH
H OOH
+R•
O O
H H
Malondialdehyde
Endoperoxide
Hydroperoxide
ROOH

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DOI: 10.3109/10715762.2013.836275

REVIEW ARTICLE

The MnSOD Ala16Val SNP: Relevance to human diseases and interaction with environmental factors

G. Bresciani[1], I. B. M. Cruz[1], J. A. de Paz[2], M. J. Cuevas[2] & J. González-Gallego[2]

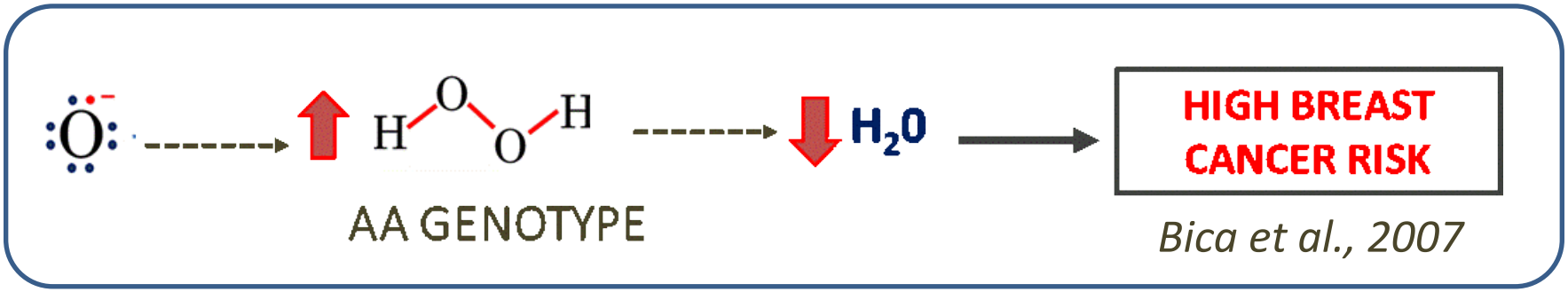
VV is associated with

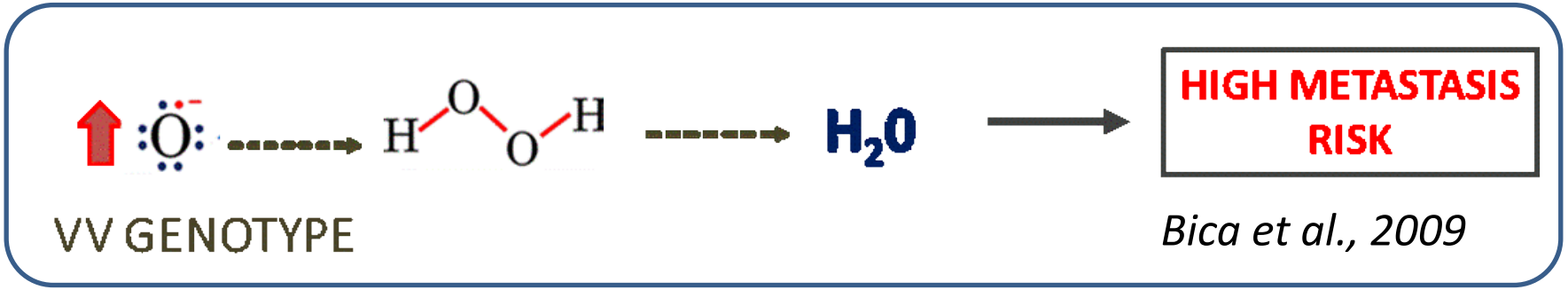
High LDL oxidizedd levels (Gottlieb et al., 2005)
Obesity (Montano et al., 2009)
Hypercholesterolemia (Duarte et al., 2010)
High level of inflammatory biomarkers (Montano et al., 2012)
mRNA SOD2 downregulation after intense exercise (Bresciani et al., 2012)

AA is associated with

High immunosenescence biomarkers (Taufer et al., 2005)
High DNA damage (Taufer et al., 2005)
Breast and Prostate cancer risk (Taufer et al., 2005; Bica et al., 2008

THE SUPEROXIDE-HYDROGEN PEROXIDE IMPACT CHANGES ACCORDING TO HEALTHY AND CANCER CELLS



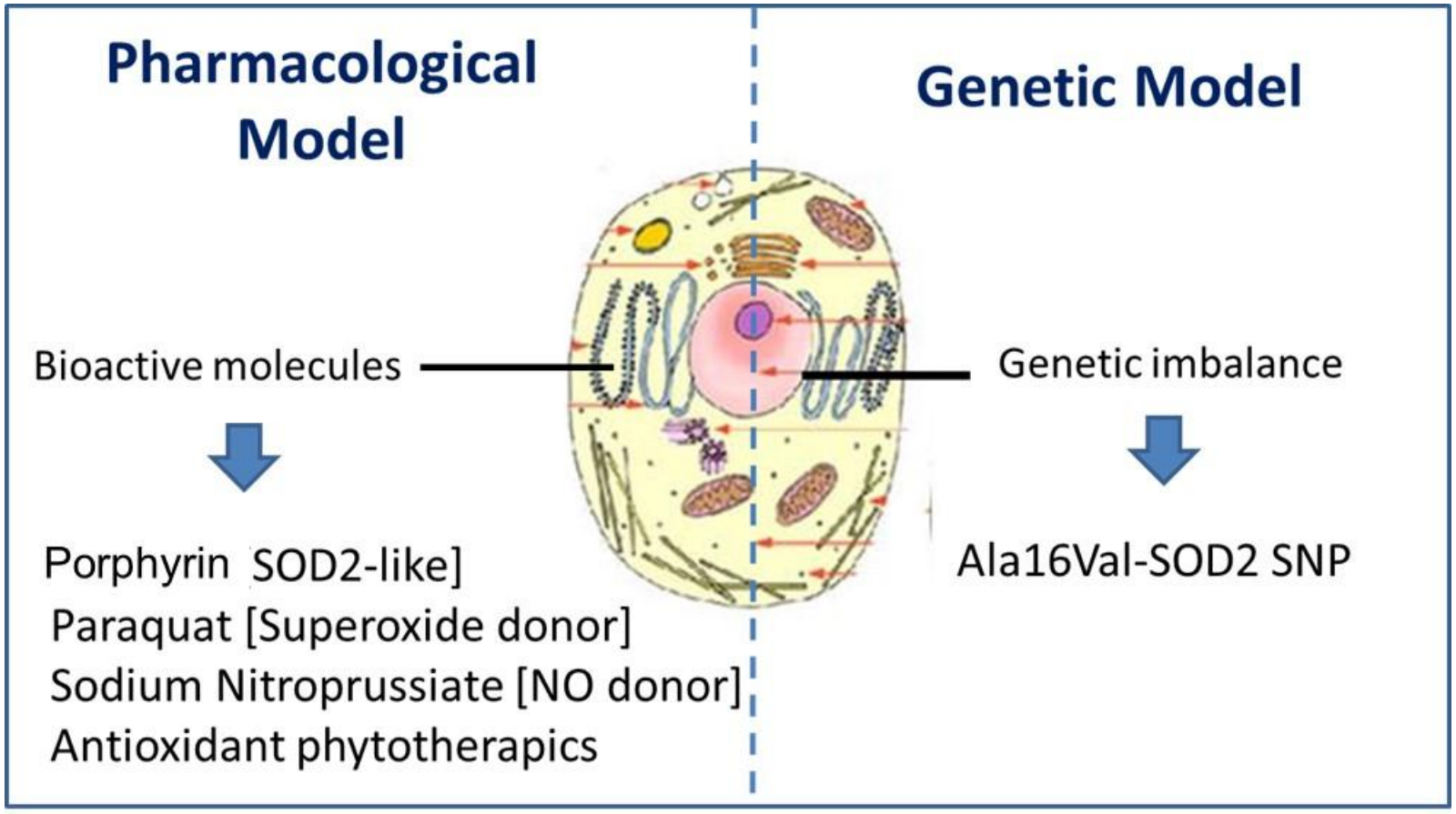




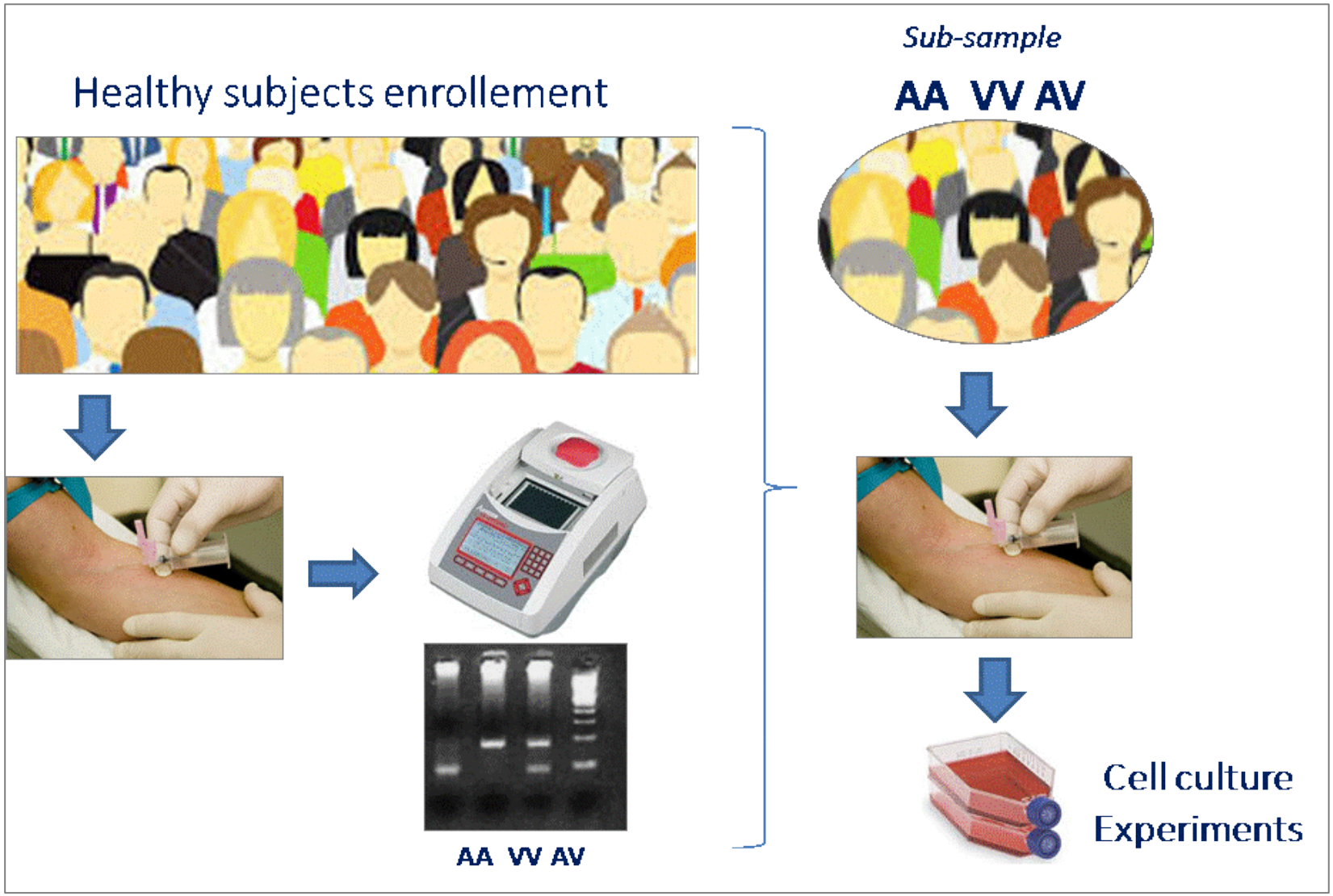
CURRENT RESEARCH FOCUS

To investigate de superoxide-hydrogen peroxide balance coordinated by SOD2 on cell function and aging

EXPERIMENTAL MODELS TO INVESTIGATE SUPEROXIDE-PEROXIDE HYDROGEN BALANCE



The *in vitro* SOD2-SNP model



LESSONS FROM IN VITRO SOD2-SNP STUDIES

POTENTIAL TOXICOGENETIC EFFECT

AA-PBMCs present high DNA damage when exposed to UV radiation
Montagner et al. *Toxicology in vitro*, 2010

AA-PBMCS presen high citotoxic effect when exposed to methylmercury
Algarver et al., *GMR,* 2013

POTENTIAL PHARMACOGENETIC EFFECT

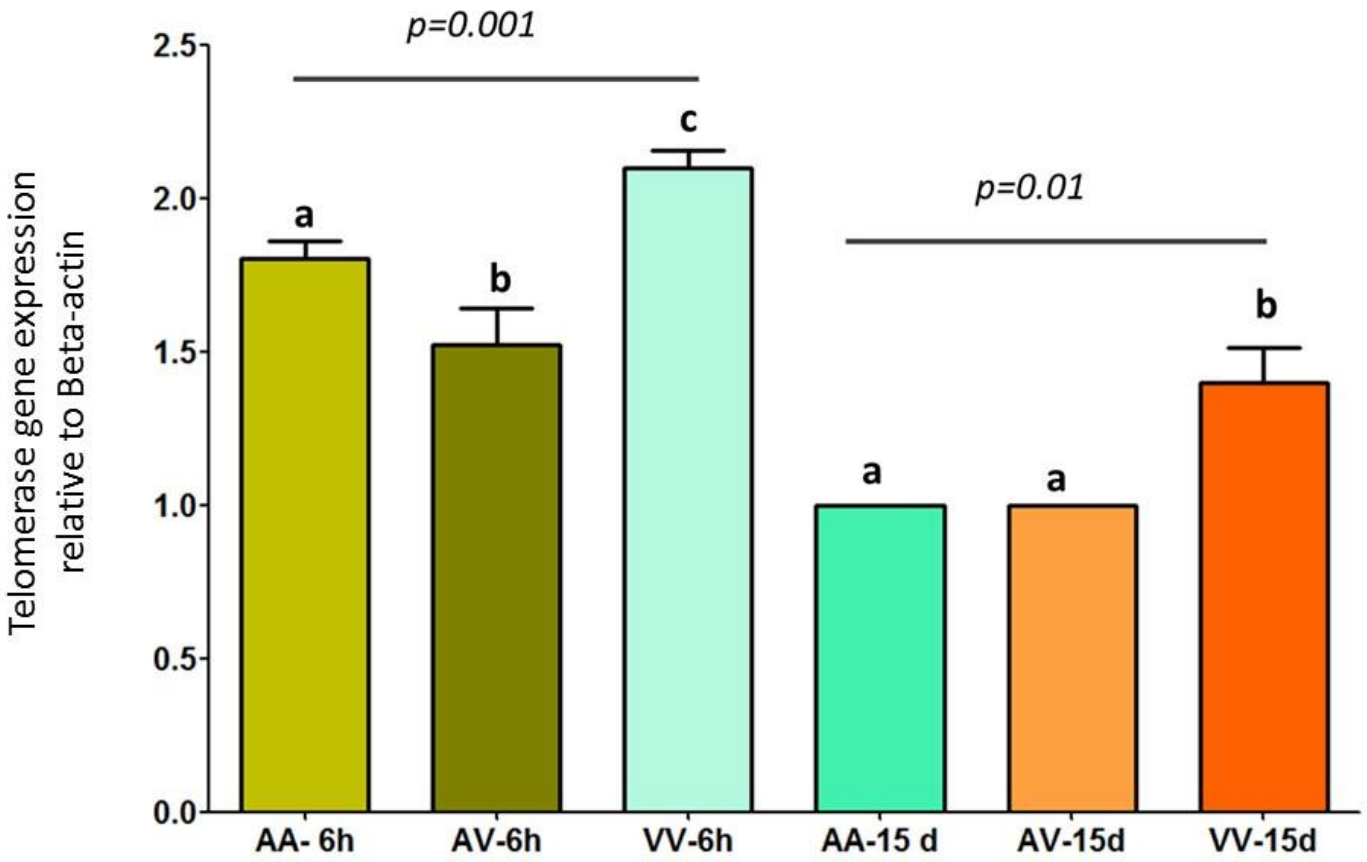
VV-PBMCs present lower antioxidant response when exposed to clomiphene citrate – Costa et al., *Reproductive Biomedicine,* 2012

VV-PBMCs present high inflammatory biomarkers when exposed to methotrexate – Barbisan et al., *Plos One,* 2014

POTENTIAL NUTRIGENETIC EFFECT

VV-PBMCs present up-regulation of Sirtuin 1 gene with and withut resveratrol exposition (Capelleto et al., *Biogerontology*, 2015)

SOD2 AND CELL AGING



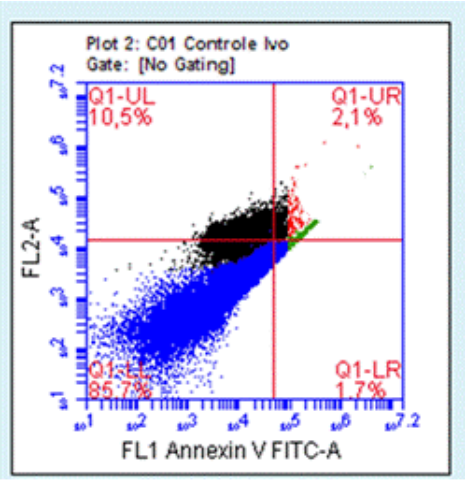
Telomerase gene expression is dependent of ASCs SOD2-genotype

Barbisan et al., 2015 – *Person Commun*

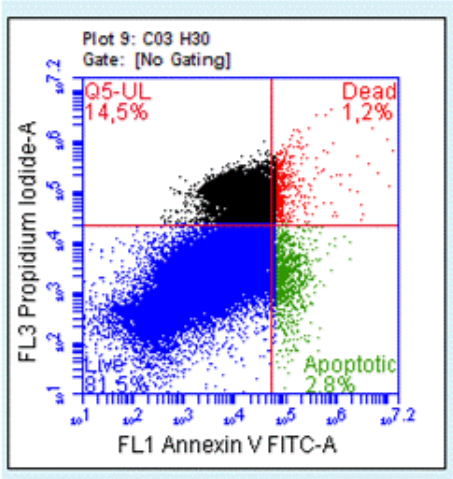
SUPEROXIDE-HYDROGENE PEROXIDE IMBALANCE ON COLORECTAL CANCER CELL LINE (HT-29)

Apoptosis induction after 24 h exposition

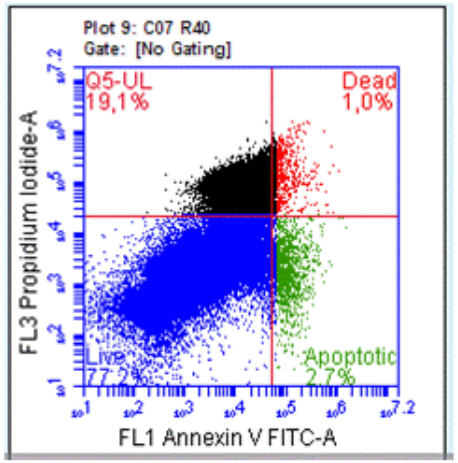
Flow cytometry PI + Annexin V



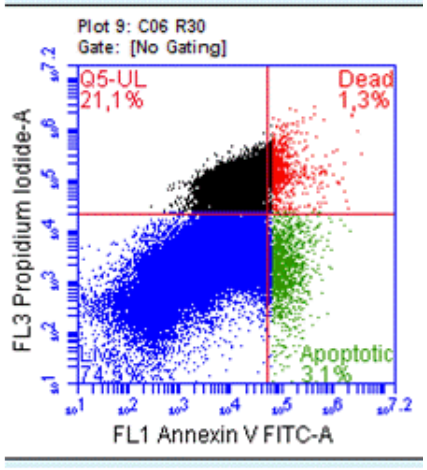
Control



Oxaliplatin 20uM



Paraquat

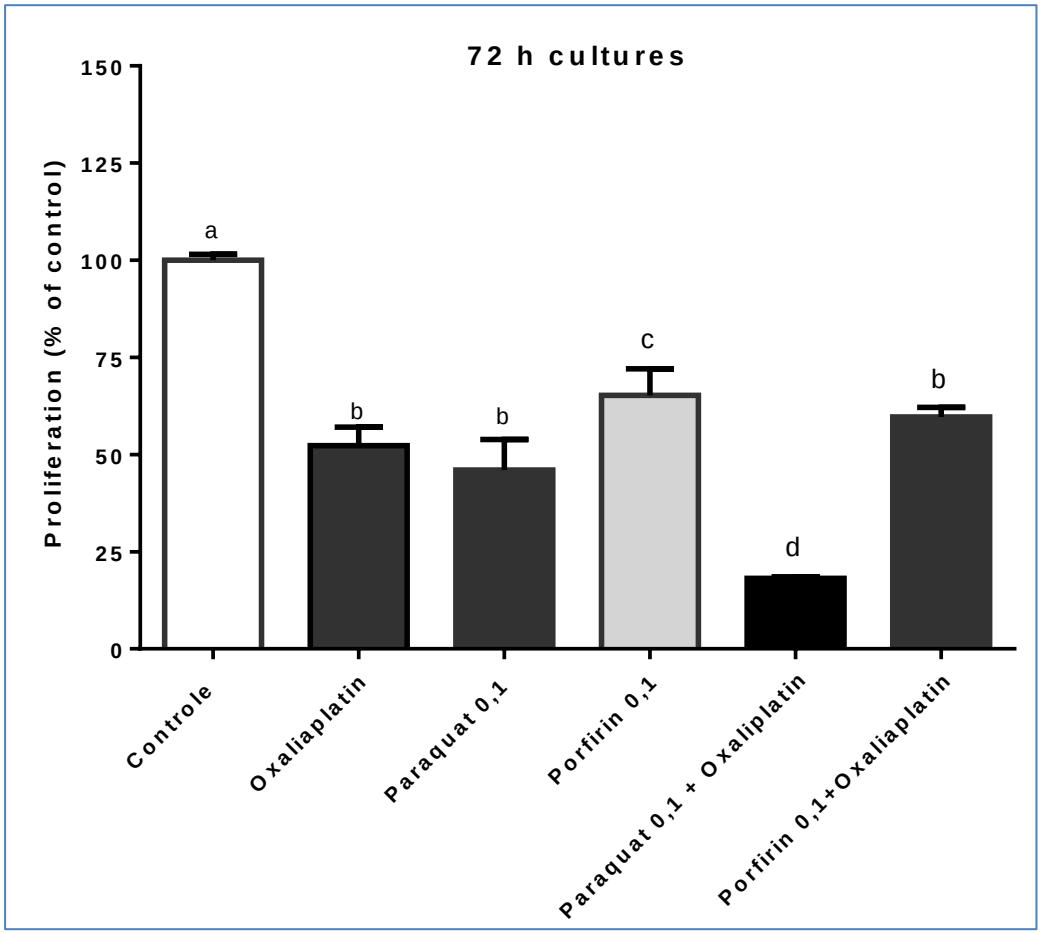


Porfirin

BOTH PHARMACOLOGICAL MIMETICS OF VV (Paraquat) and AA (Porfirin) genotypes induced apoptosis on HT-29 cells

Azzolin et al. 2015, person commun

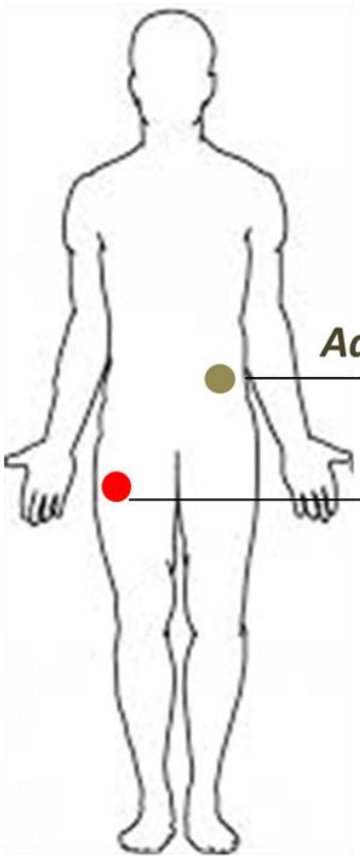
PORFIRINE (SOD2-LIKE) AND PARAQUAT (SUPEROXIDE DONNOR) ON APOPTOSIS INDUCTION OF COLORECTAL CANCER CELL LINE (HT-29)



Paraquat increased the antiproliferactive effectS of Oxaliplatin

Azzolin et al. 2015, person commun

Regenerative Medicine



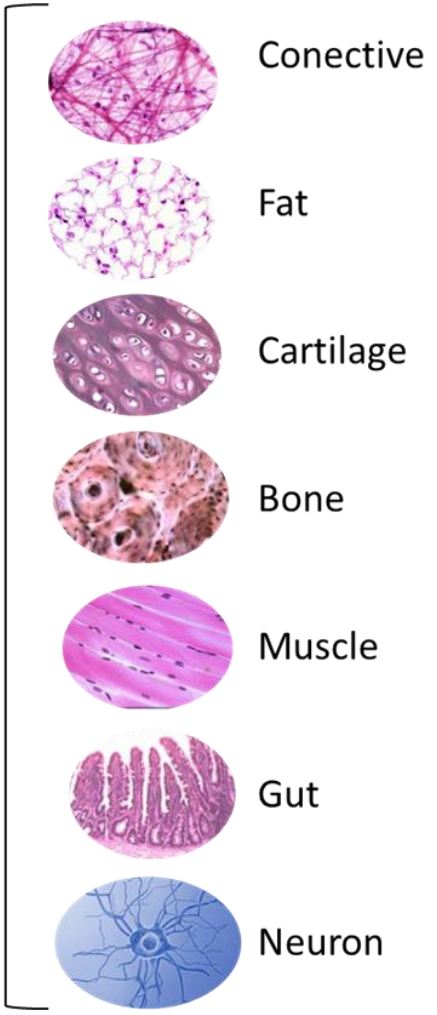
Adult Body

Adipocyte tissue

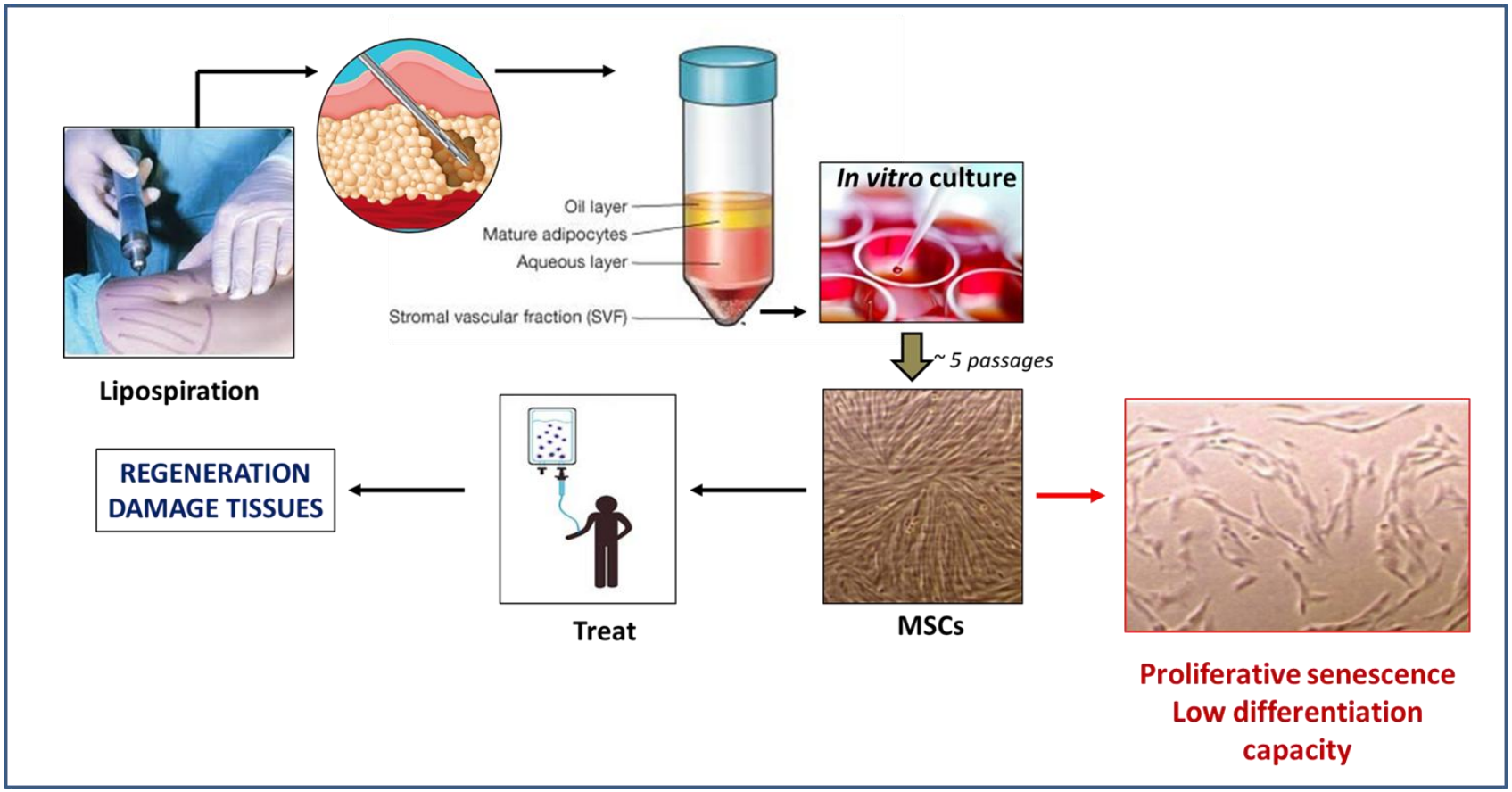
Bone marrow



Mesenchymal Stem Cells (MSC)



Regenerative Medicine: challenges



The *in vitro* stress conditions lead to REPLICATIVE SENESCENCE

The H_2O_2 is a key molecule on MSCs biology

Citation: Cell Death and Disease (2011) 2, e213; doi:10.1038/cddis.2011.96
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www.nature.com/cddis

Review

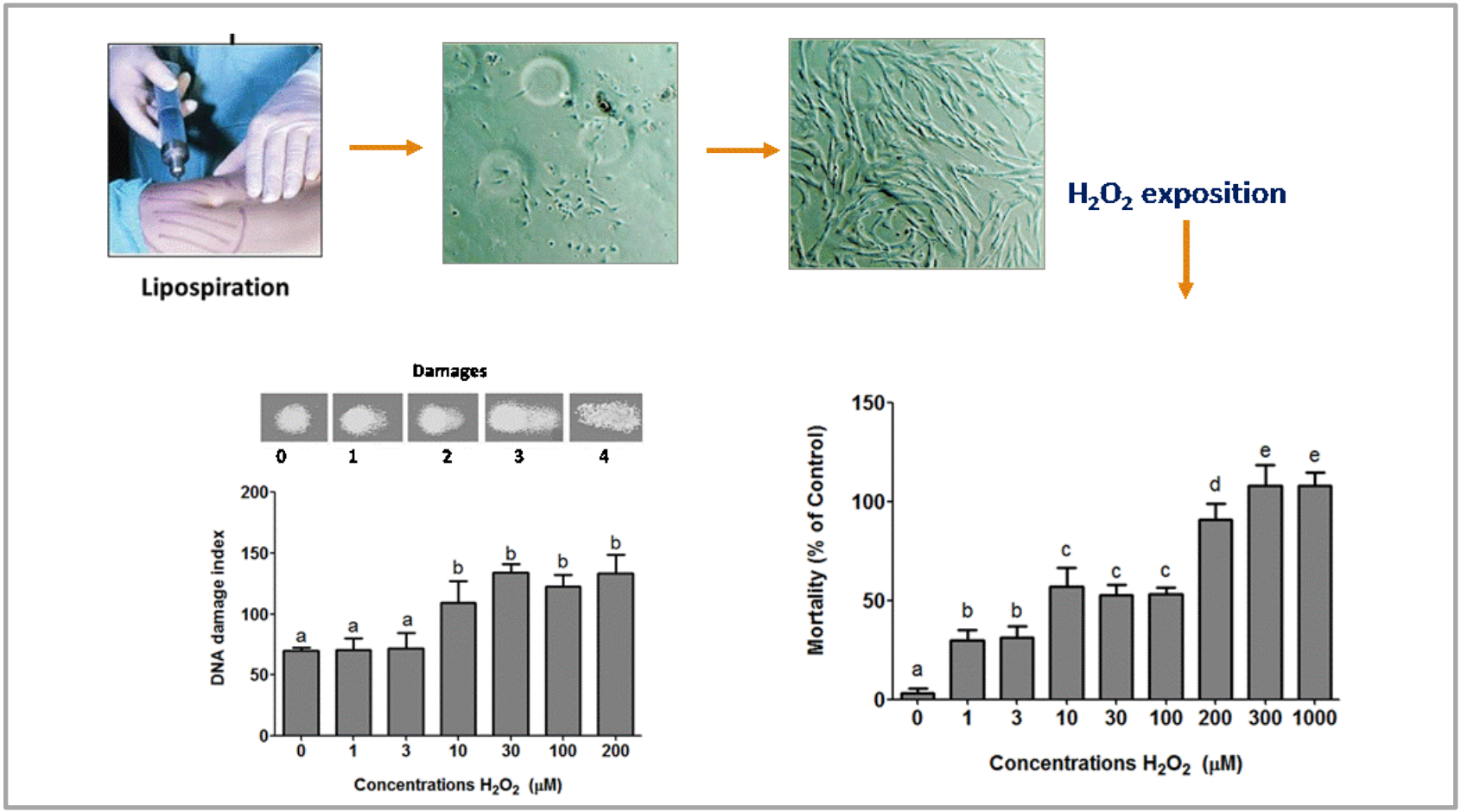
Hydrogen peroxide: a Jekyll and Hyde signalling molecule

DR Gough[1] and TG Cotter*,[1]

The H_2O_2 plays a dual role on MSCs

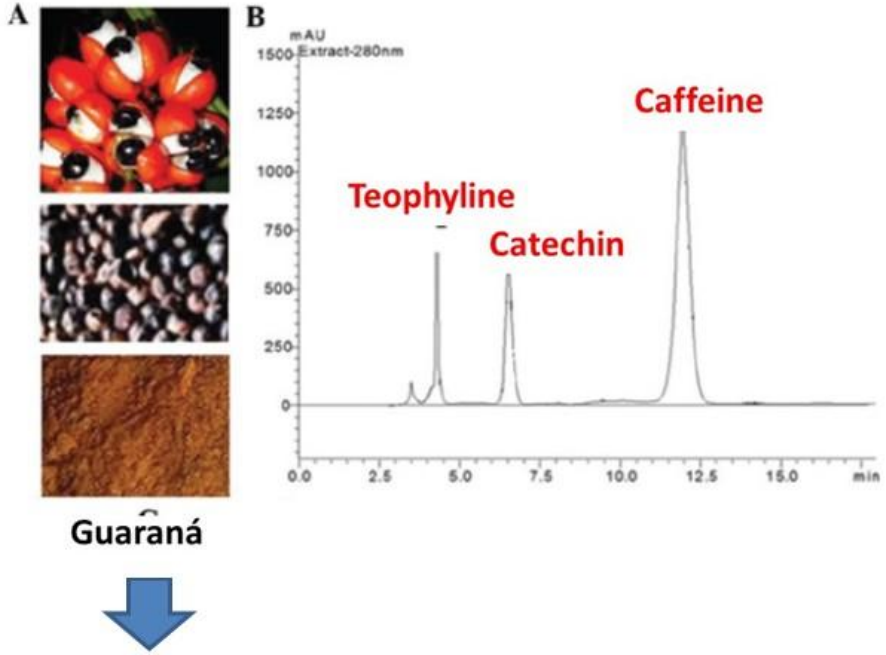
- **Proliferative cell estimulation**
- **DNA damage – apoptosis induction**

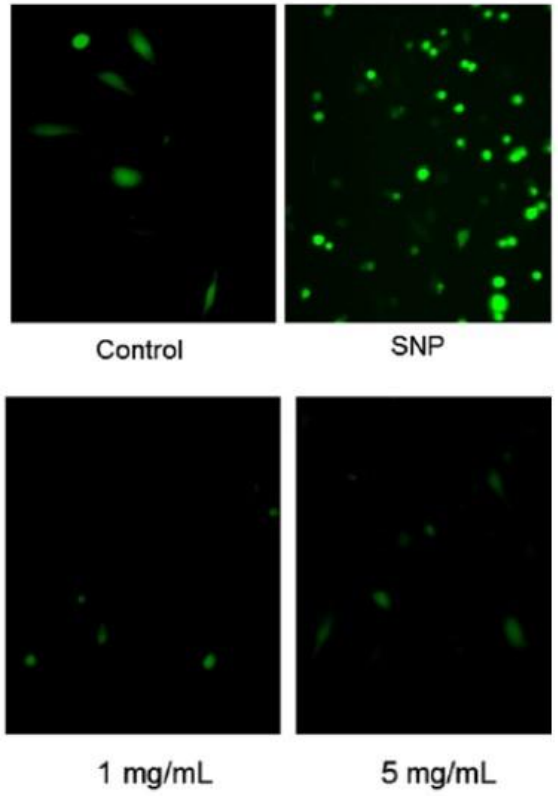
The H_2O_2 induced DNA damage and apoptosis of Adipocyte Stem Cells (Ascs) obtained from human lipoaspirates



Machado et al. 2015, Person commun.

RESPONSE OF STEM CELLS TO FOODS THAT MODULATE DIFFERENTIALLY SOD2





Experimental model: NIH-3T3 cell line

Paullinia cupana modulates nitric oxide levels presentes SOD-like activity

Bittencourt et al., Food Chem Toxicol, 2013

RESPONSE OF STEM CELLS TO FOODS THAT MODULATE

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

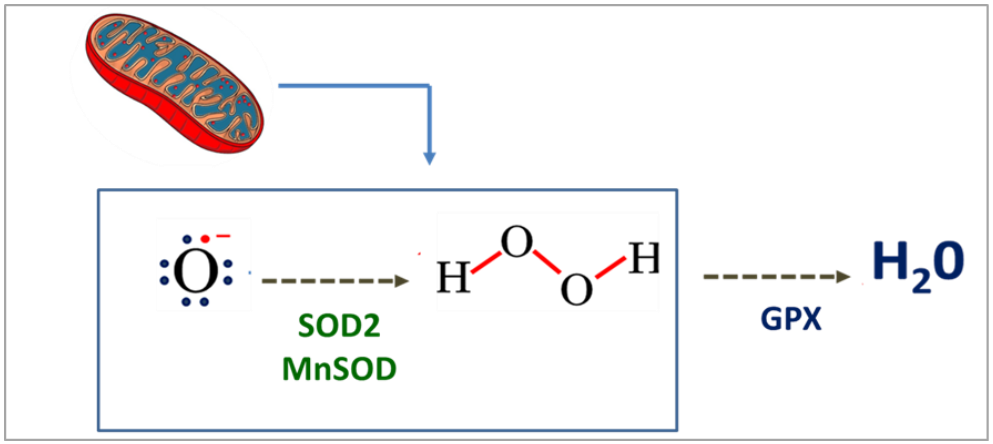


Guaraná (*Paullinia cupana*) improves the proliferation and oxidative metabolism of senescent adipocyte stem cells derived from human lipoaspirates

Alencar Kolinski Machado [a], Francine Carla Cadoná [b], Verônica Farina Azzolin [a], Eduardo Bortoluzzi Dornelles [b], Fernanda Barbisan [a], Euler Esteves Ribeiro [c], Maria Fernanda Mânica-Cattani [c], Marta Maria Medeiros Frescura Duarte [c], José Raul Pinto Saldanha [c], Ivana Beatrice Mânica da Cruz [a,b,c,*]

Phytotherapics extracts that modulate differentially SOD2 gene/acticity improved proliferation of senescent ASCs

EFFECT OF SUPEROXIDE-HYDROGEN PEROXIDE IMBALANCE ON CELL BIOLOGY



- Proliferation
- Differentiation
- Senescence
- Environmental Response

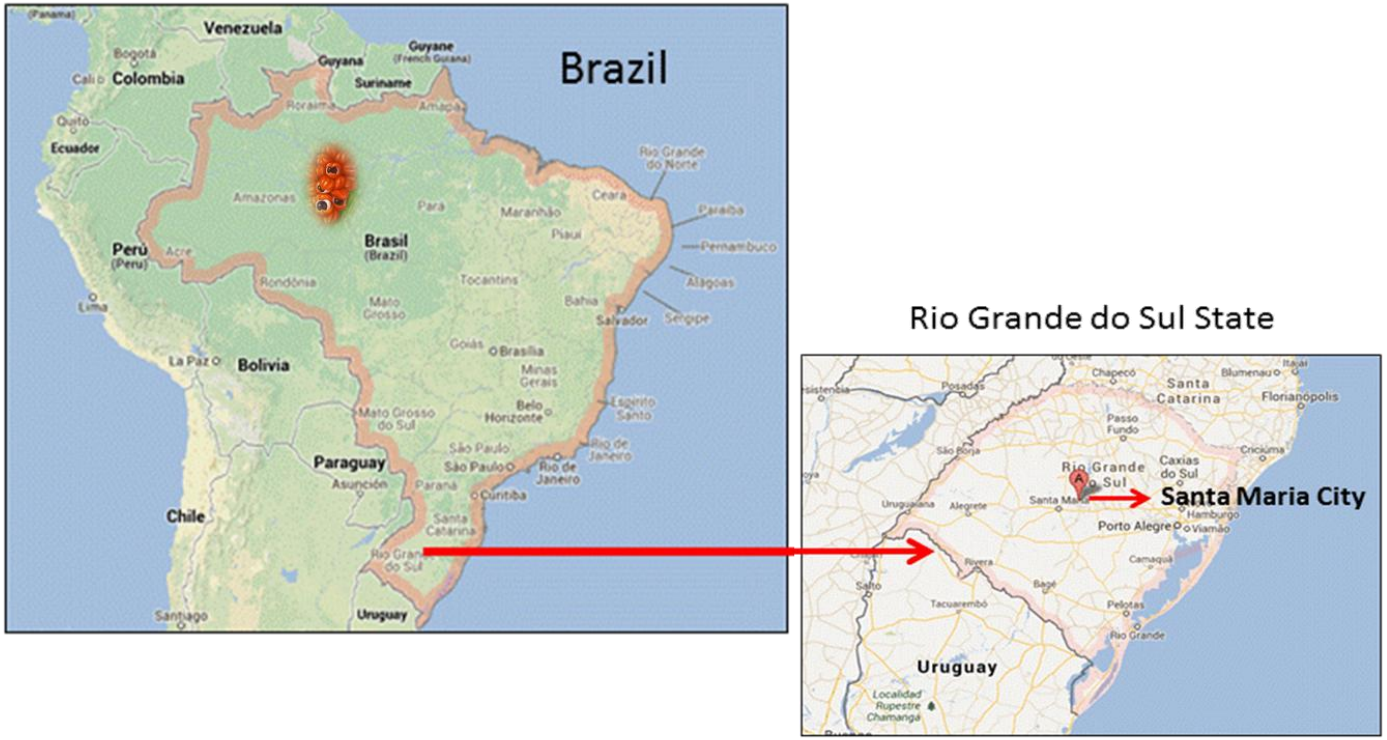
Effect on MSCs biology (Proliferation? Differentiation? Senescence?)
Effect on Cancer cell (Chemotherapy drug resistance?)

CONCLUSION AND FUTURE DIRECTIONS

Superoxide-hydrogen peroxide balance and not only hydrogen peroxide is relevant to cell proliferation and senescence.

The concomitant management of superoxide-hydrogen peroxide balance could be an important tool to improve the safety and efficiency of stem cell regenératives therapies.

Federal University of Santa Maria



Thank you very much!

ibmcruz@hotmail.com