Sweet, Sticky, and Healthy – using metabolomics to develop a 'green' procotol for polyphenolics extraction from wine grape pomace

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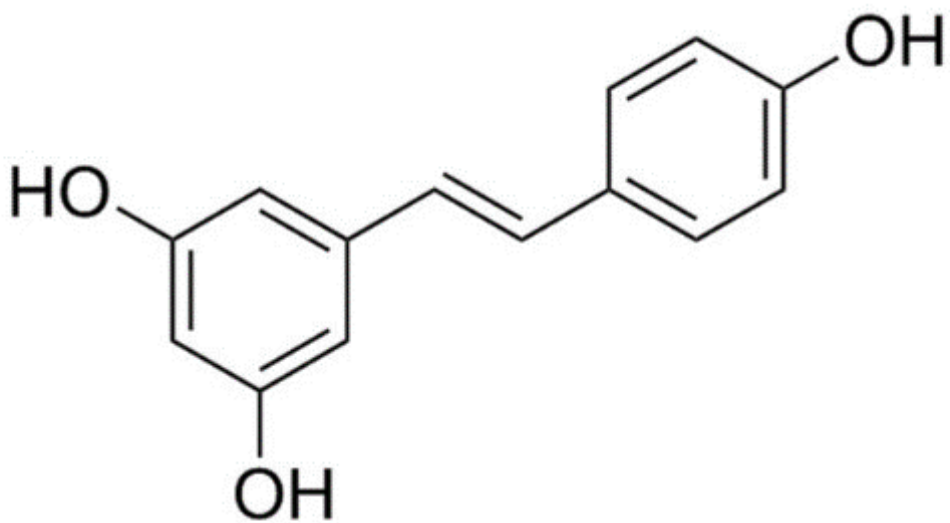
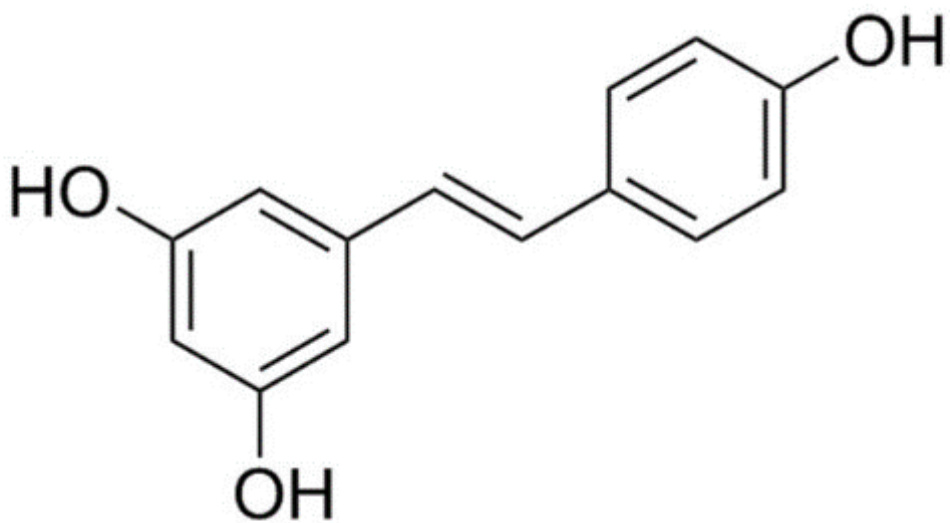


Resveratrol:

- Why
- Where
- How



Resveratrol:

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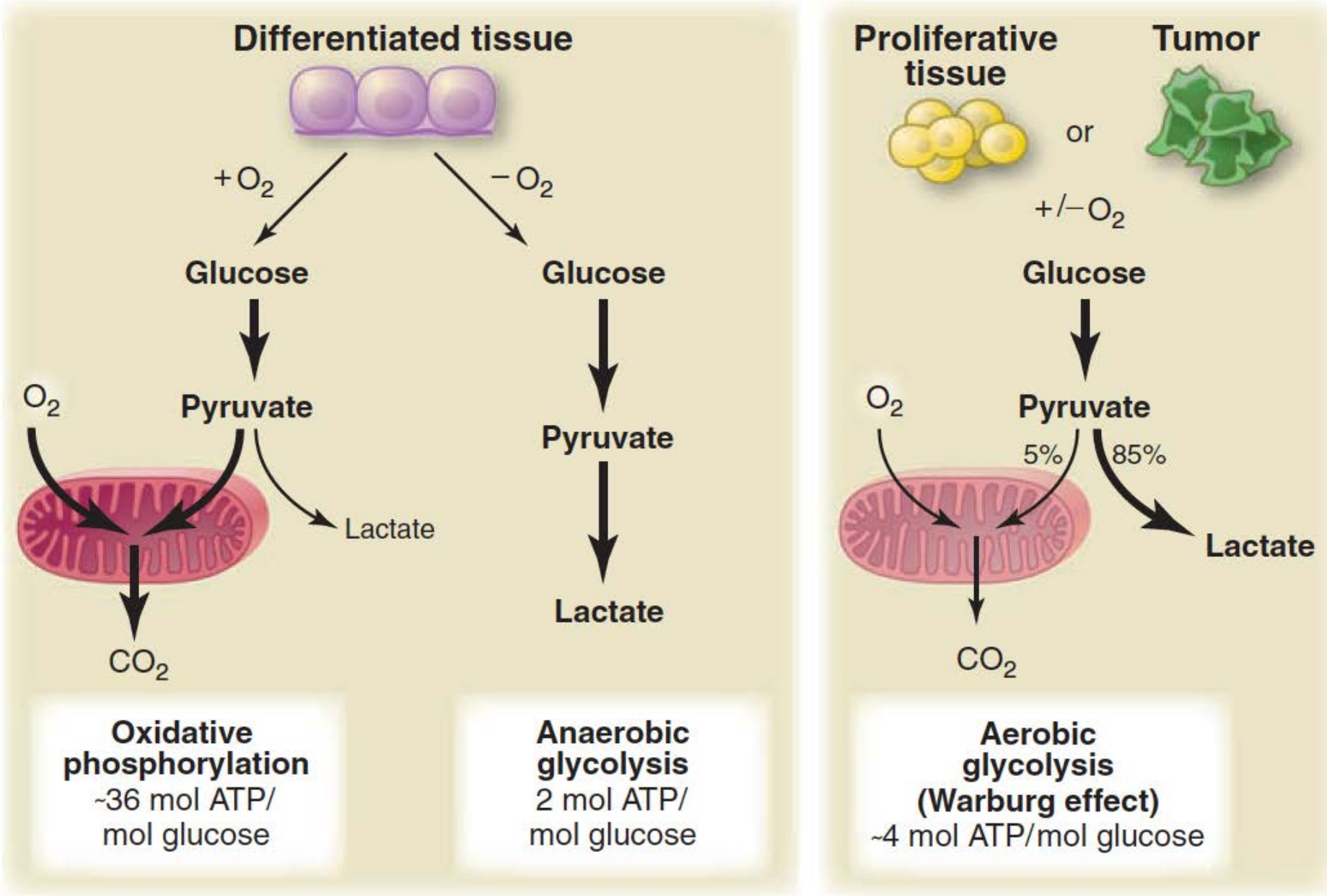
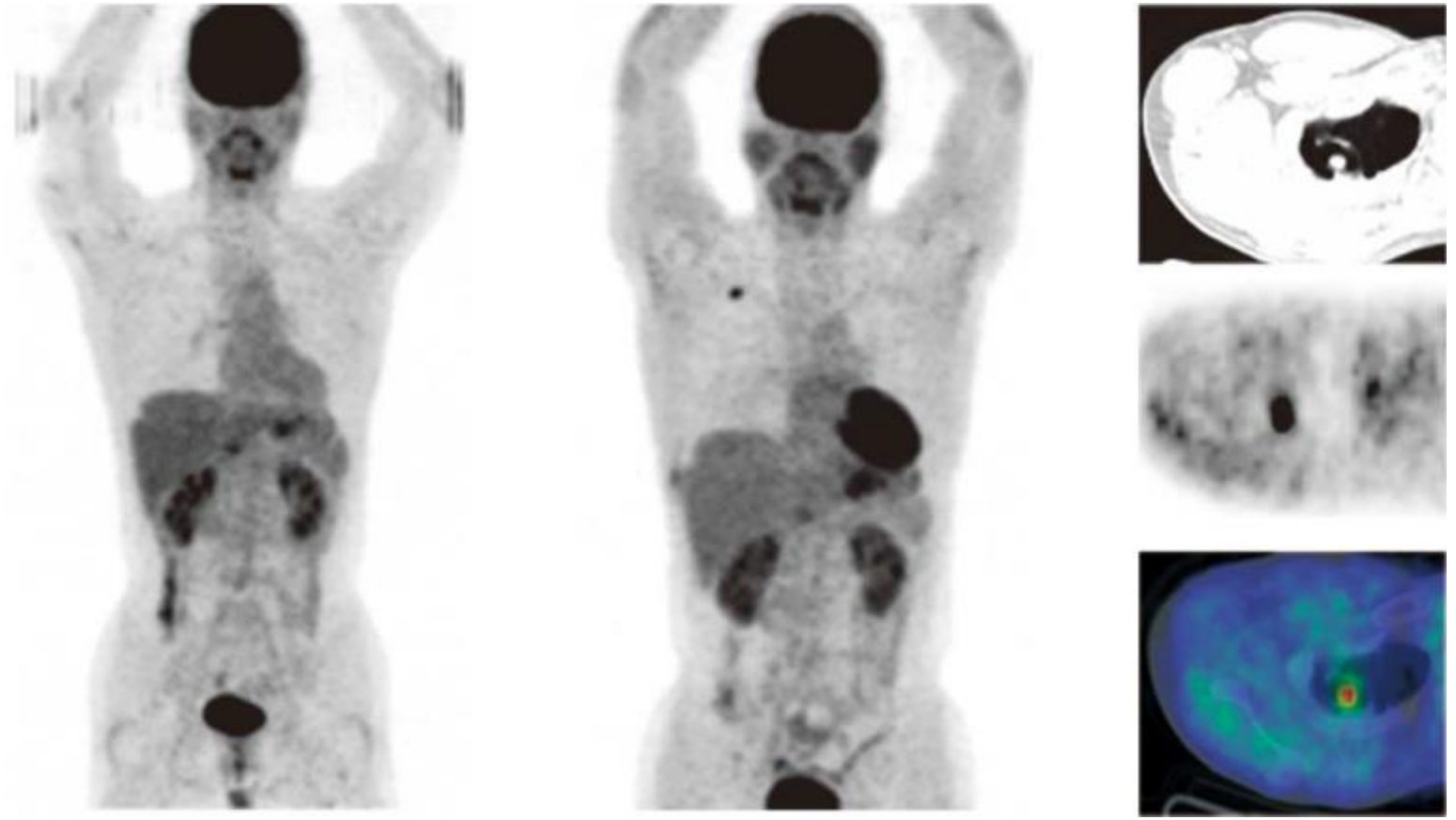
The Warburg Effect:

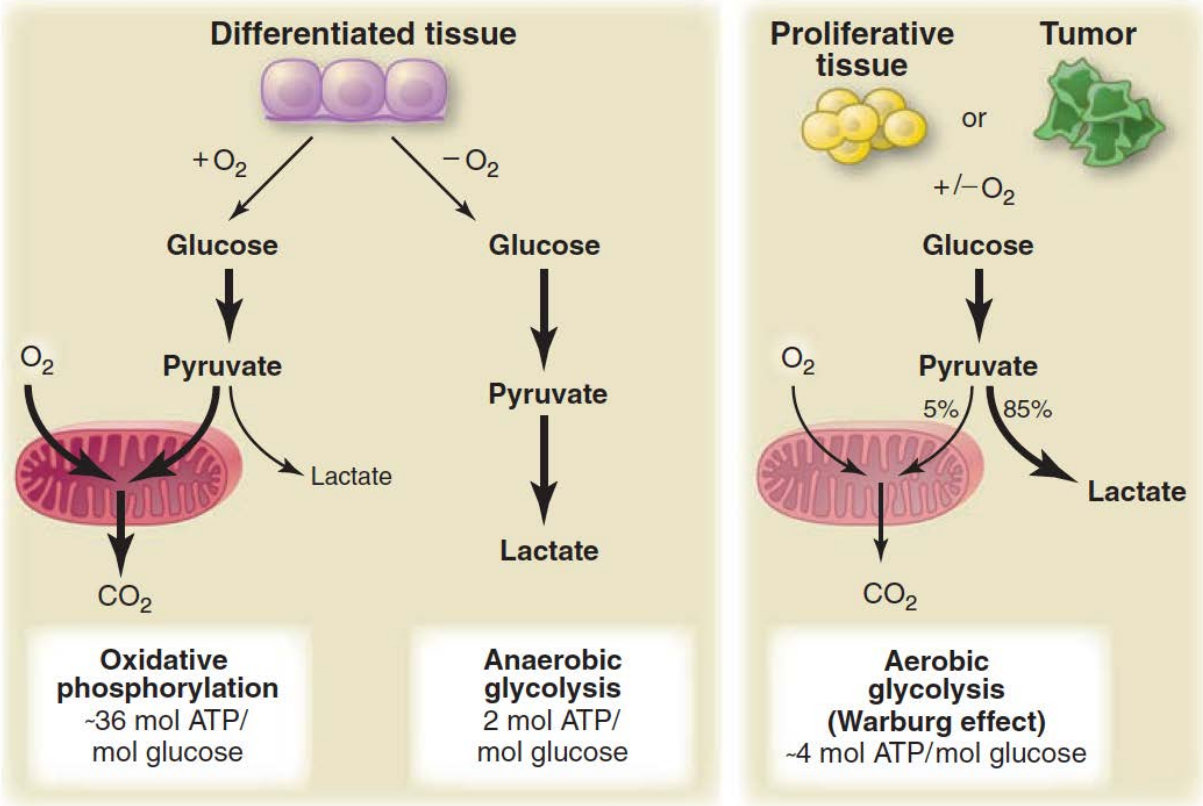


The Warburg Effect is exploited in the ^{18}F-deoxyglucose PET scan to detect cancers



One strategy for slowing growth of cancer cells is to target the Warburg Effect

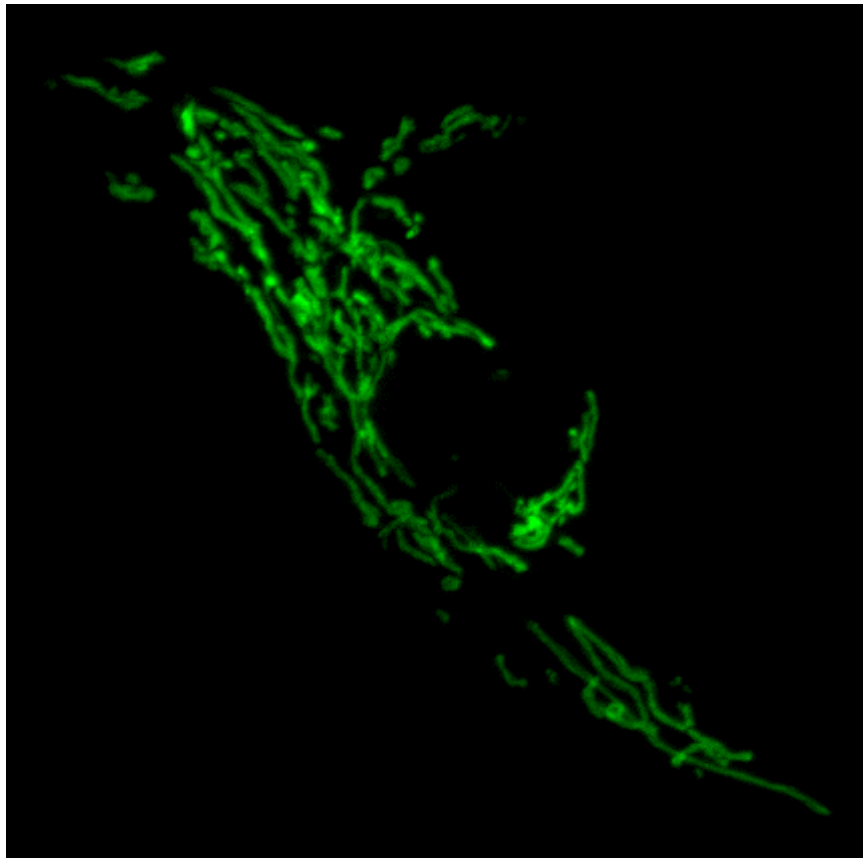
- Inhibit glucose fermentation / stimulate mitochondrial oxidative phosphorylation to slow cancer growth

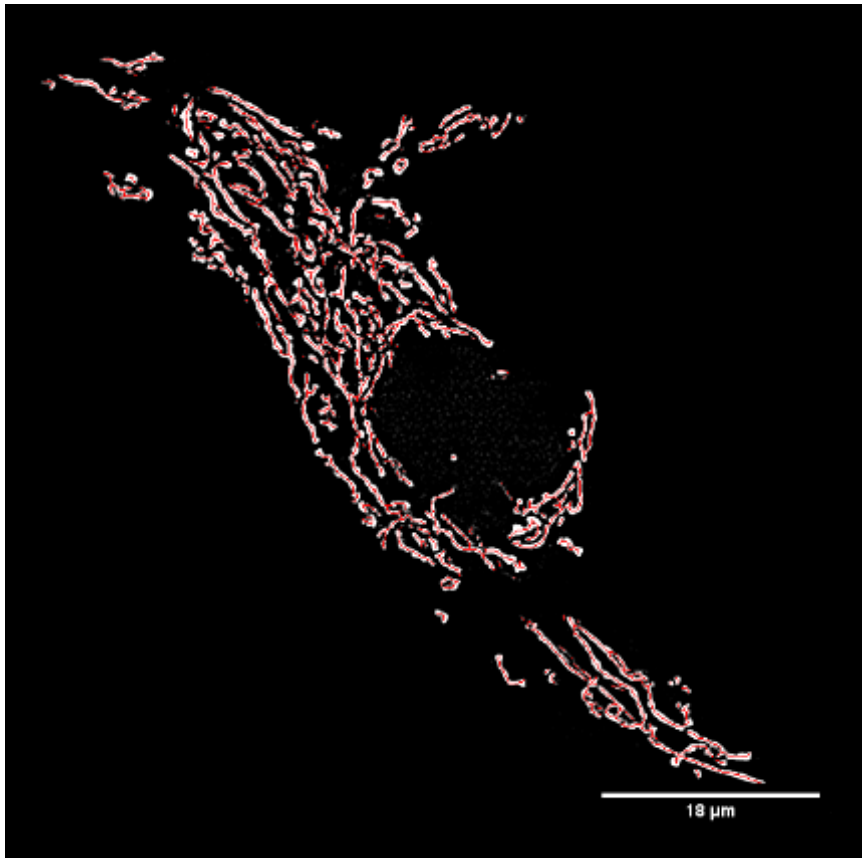


MiNA

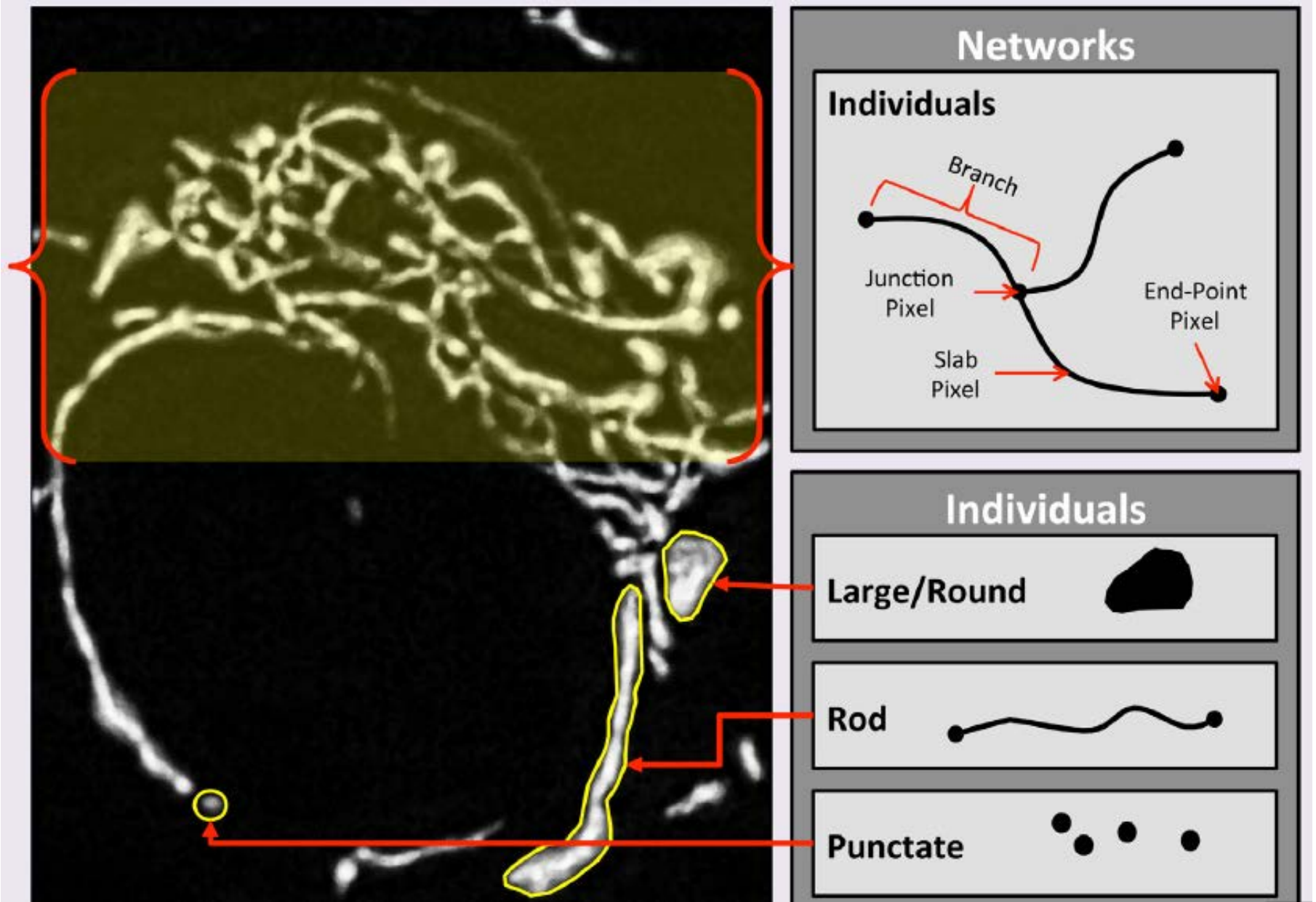
A simple ImageJ macro tool for analyzing mitochondrial network morphology in mammalian cell culture

Andrew J. Valente[a,*], Lucas A. Maddalena[a], Ellen L. Robb[b], Fereshteh Moradi[a], Jeffrey A. Stuart[a,*]



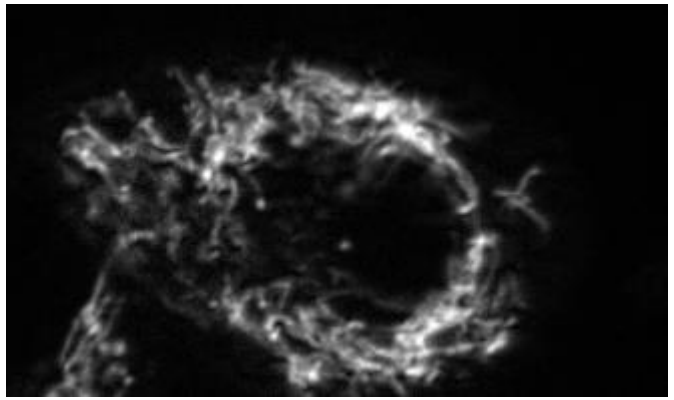


Assessing resveratrol effects on mitochondria

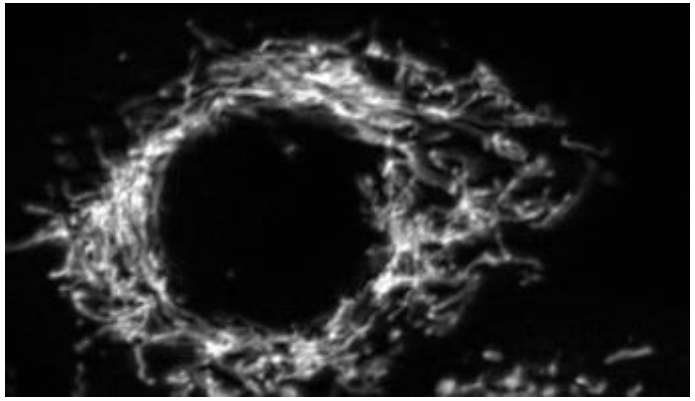


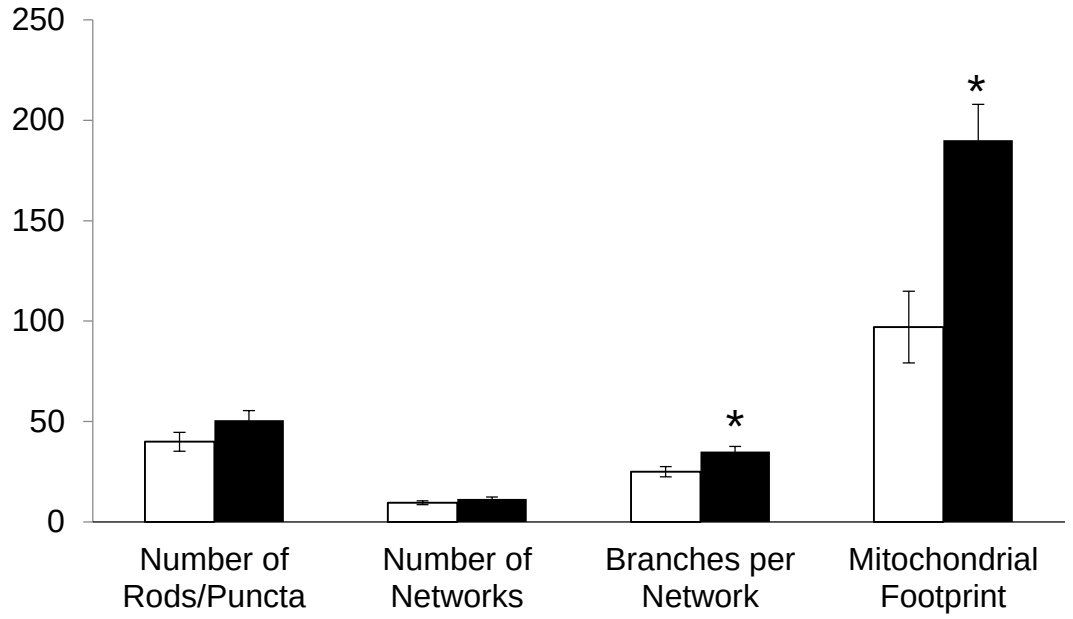
Treatment (72h) of prostate cancer cells with 10 μM resveratrol stimulates mitochondrial biogenesis and fusion

Control

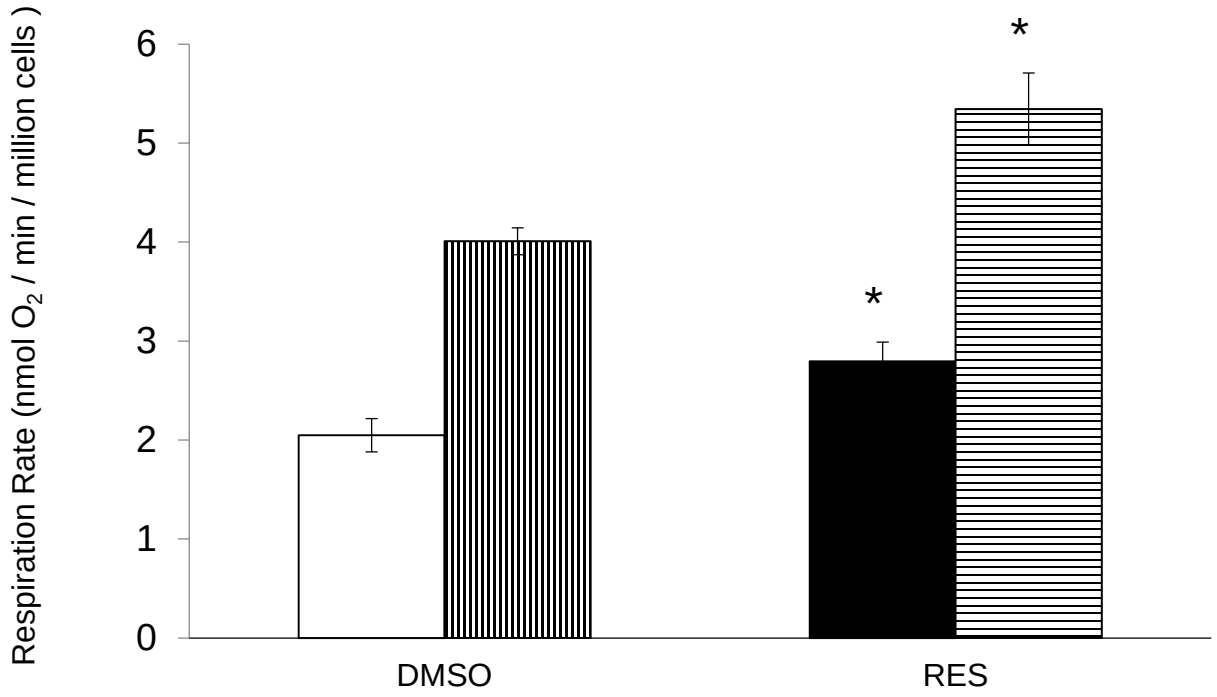


Resveratrol

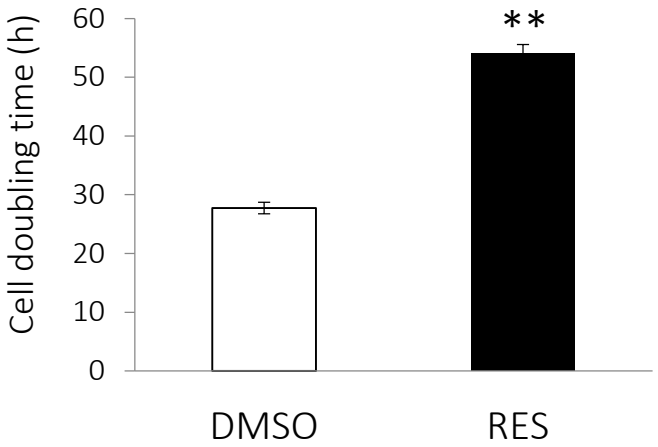


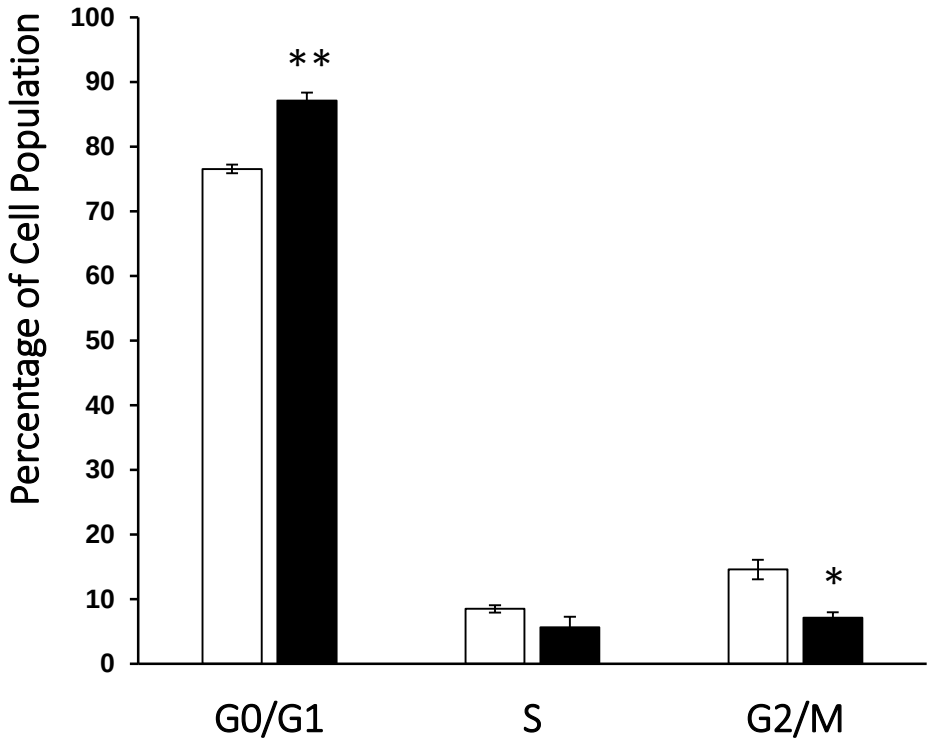


Treatment (72h) of prostate cancer cells with 10 µM resveratrol stimulates respiration



Concomitantly, resveratrol slows prostate cancer cell growth and cells accumulate in G0/G1





□ DMSO ■ RES

mitochondrial biogenesis
mitochondrial fusion
mitochondrial respiration

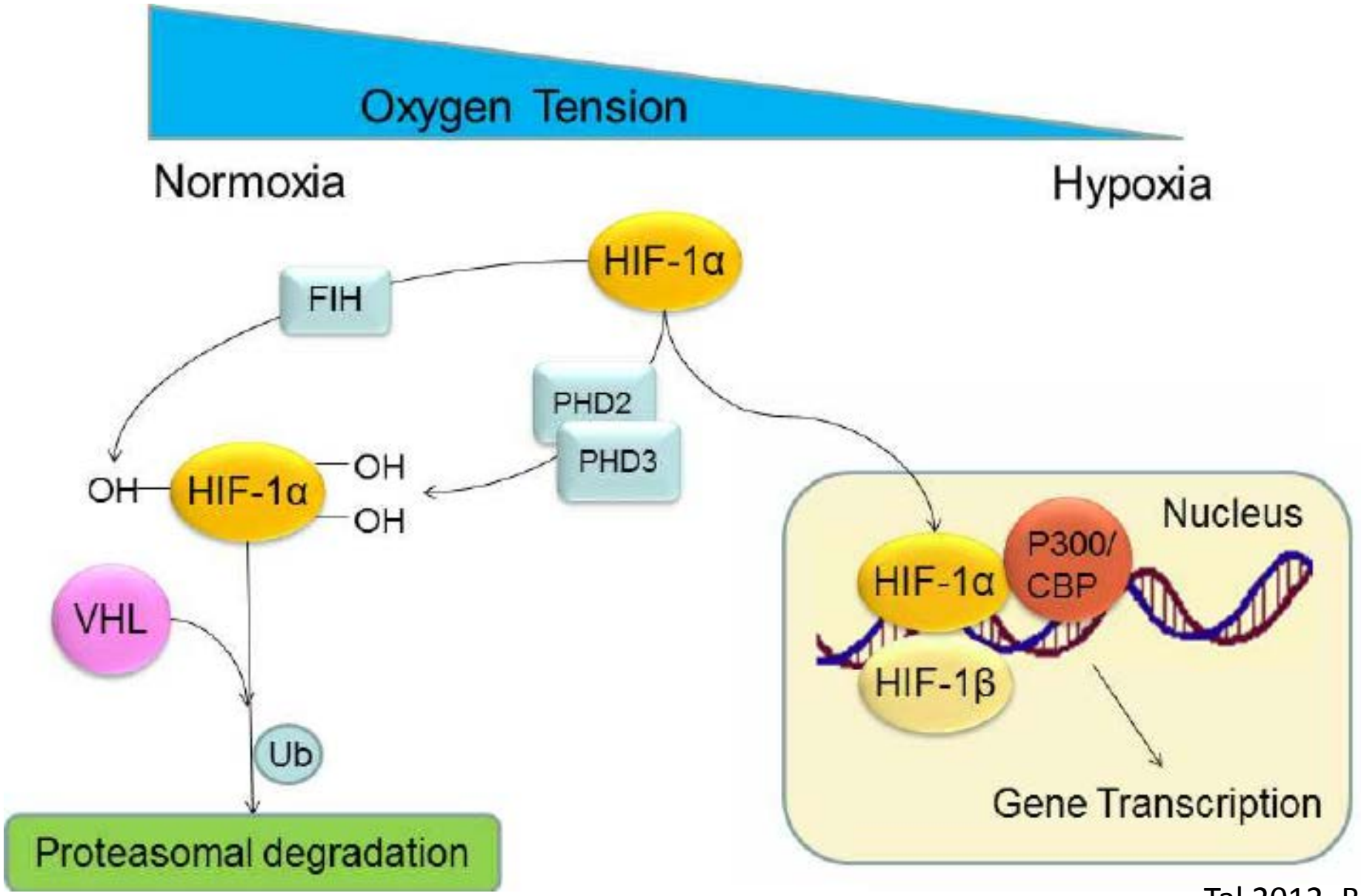
Resveratrol

cell growth

Is the shift from a glycolytic to oxidative phenotype required for resveratrol's effects on PC3 cells?

- Investigate potential role of Hypoxia Inducible Factor-1 (HIF-1), which is elevated in normoxia in some cancer cells, including PC3.

HIF-1α regulation

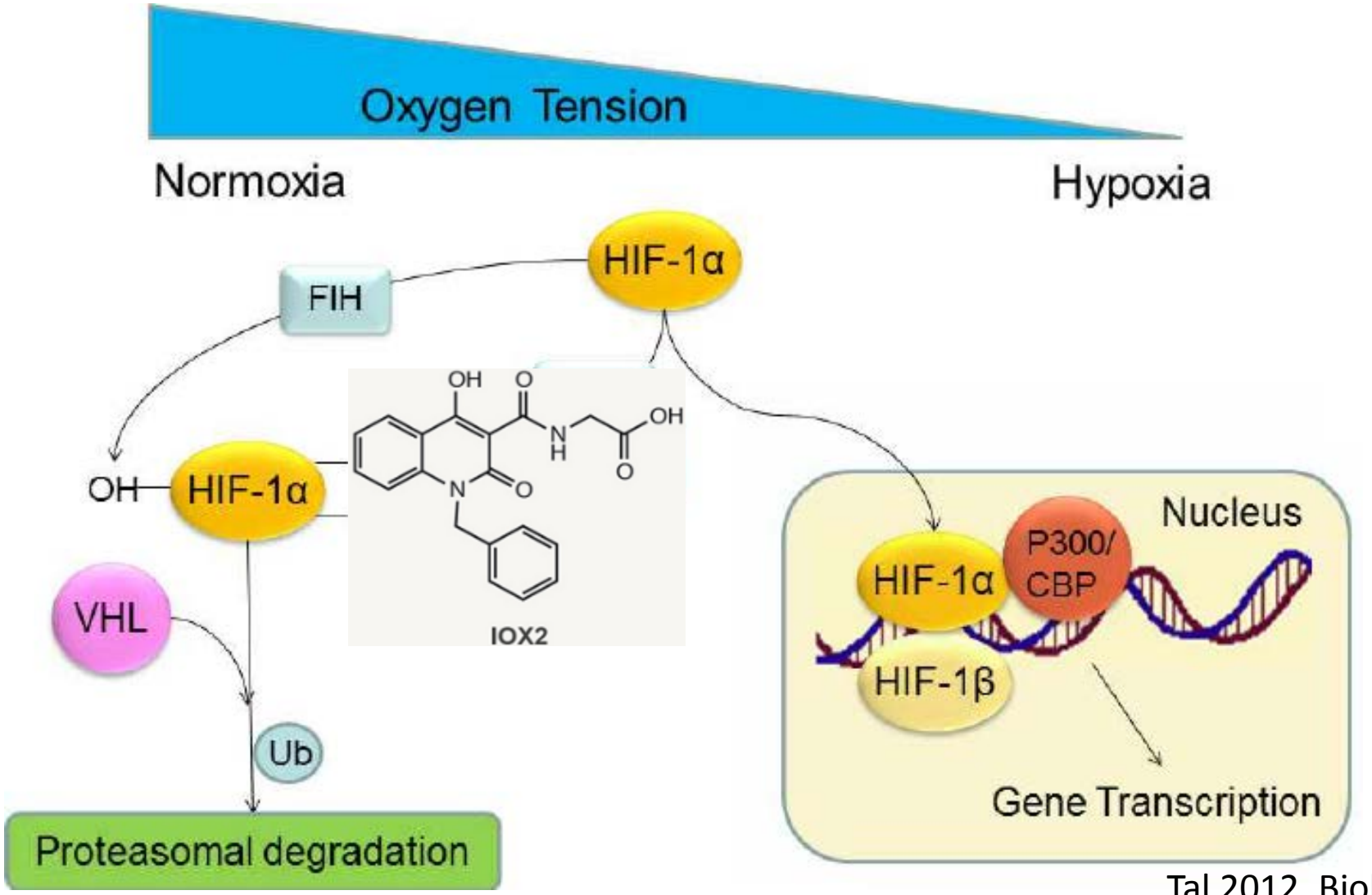


Tal 2012, Biol of Reprod.

Experimental approach:

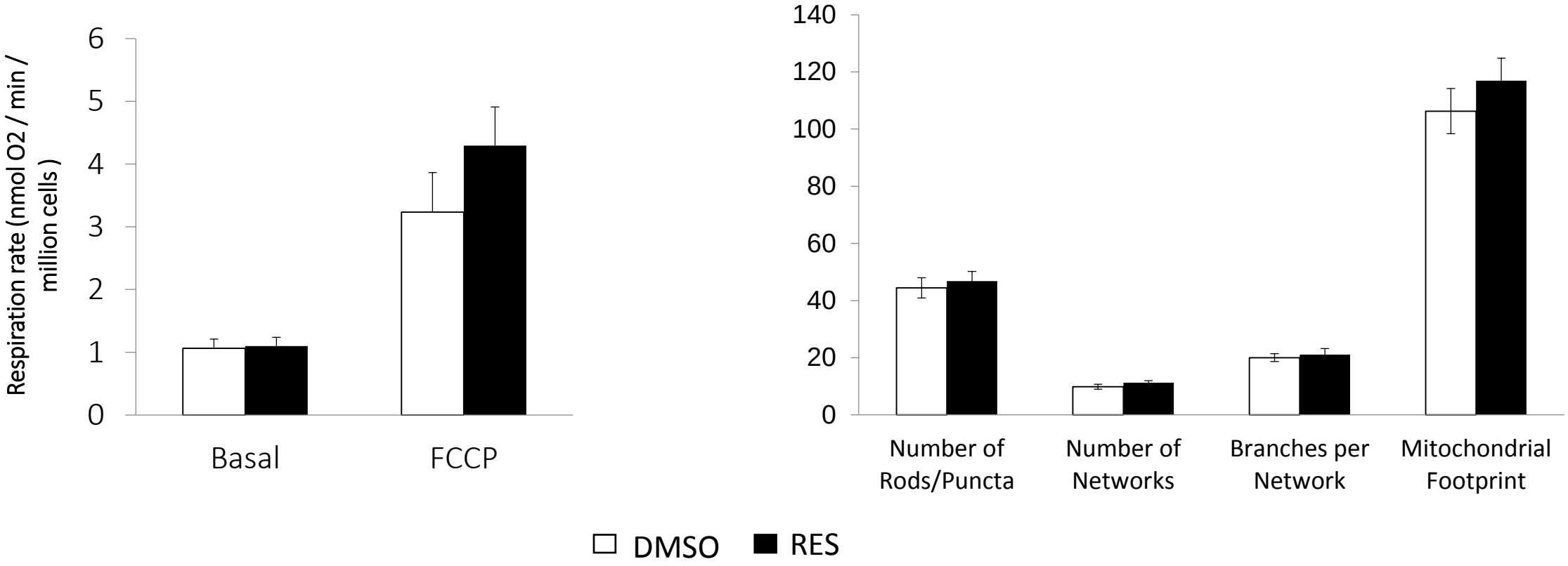
- **Stabilize HIF-1α expression by inhibiting degradation (IOX2)**

IOX2 inhibits the HIF prolyl hydroxylases (PHDs), thus stabilizing HIF-1α

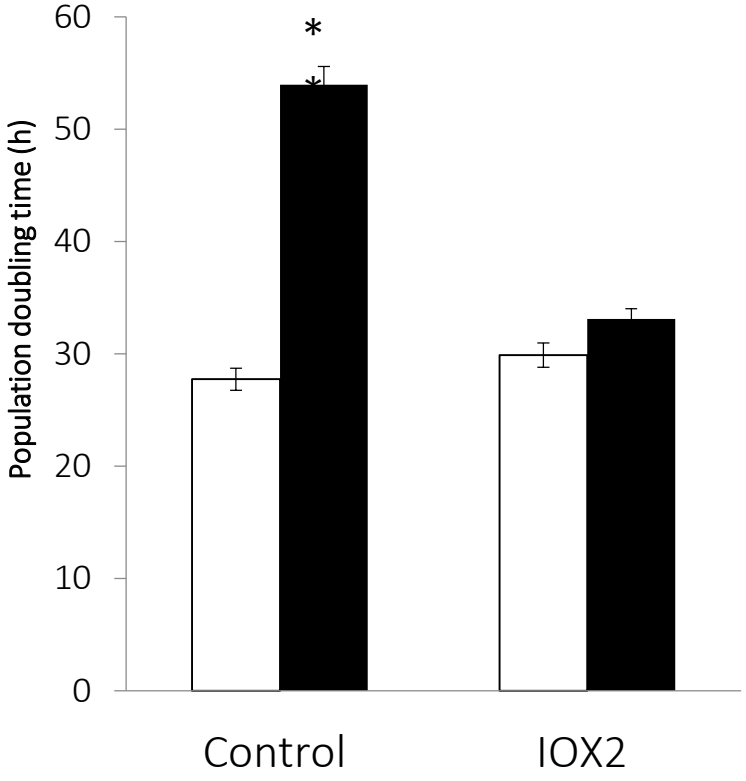


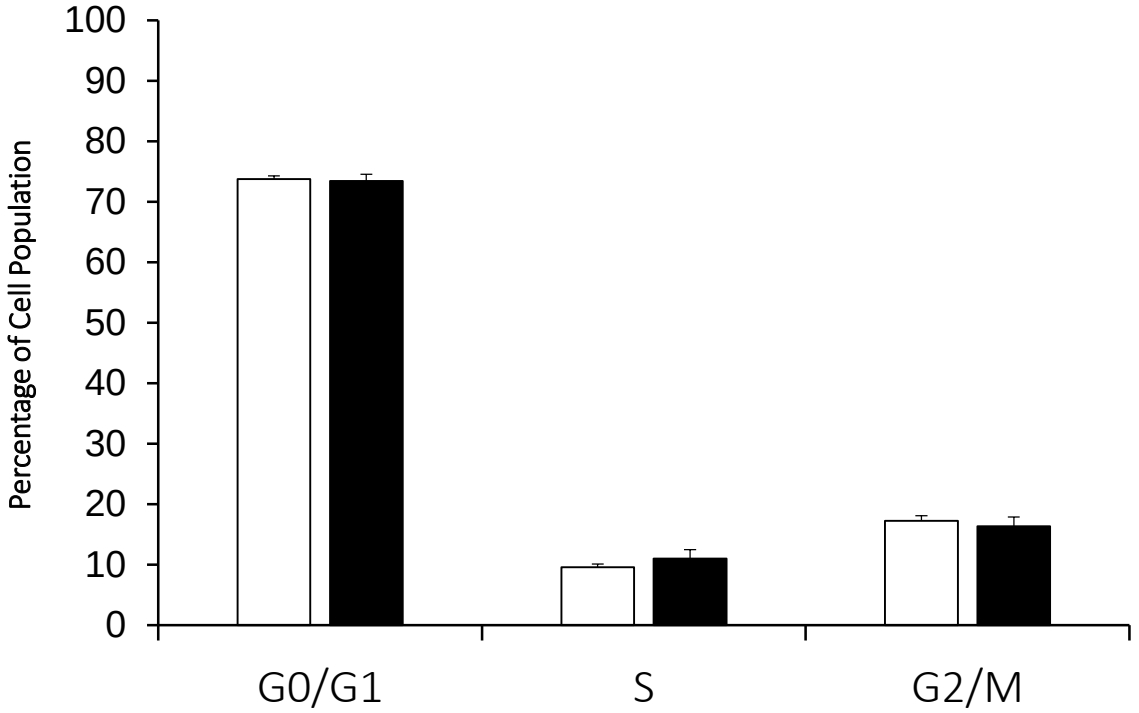
Tal 2012, Biol of Reprod.

No effects of resveratrol on mitochondrial form or function in IOX2-treated PC3 cells



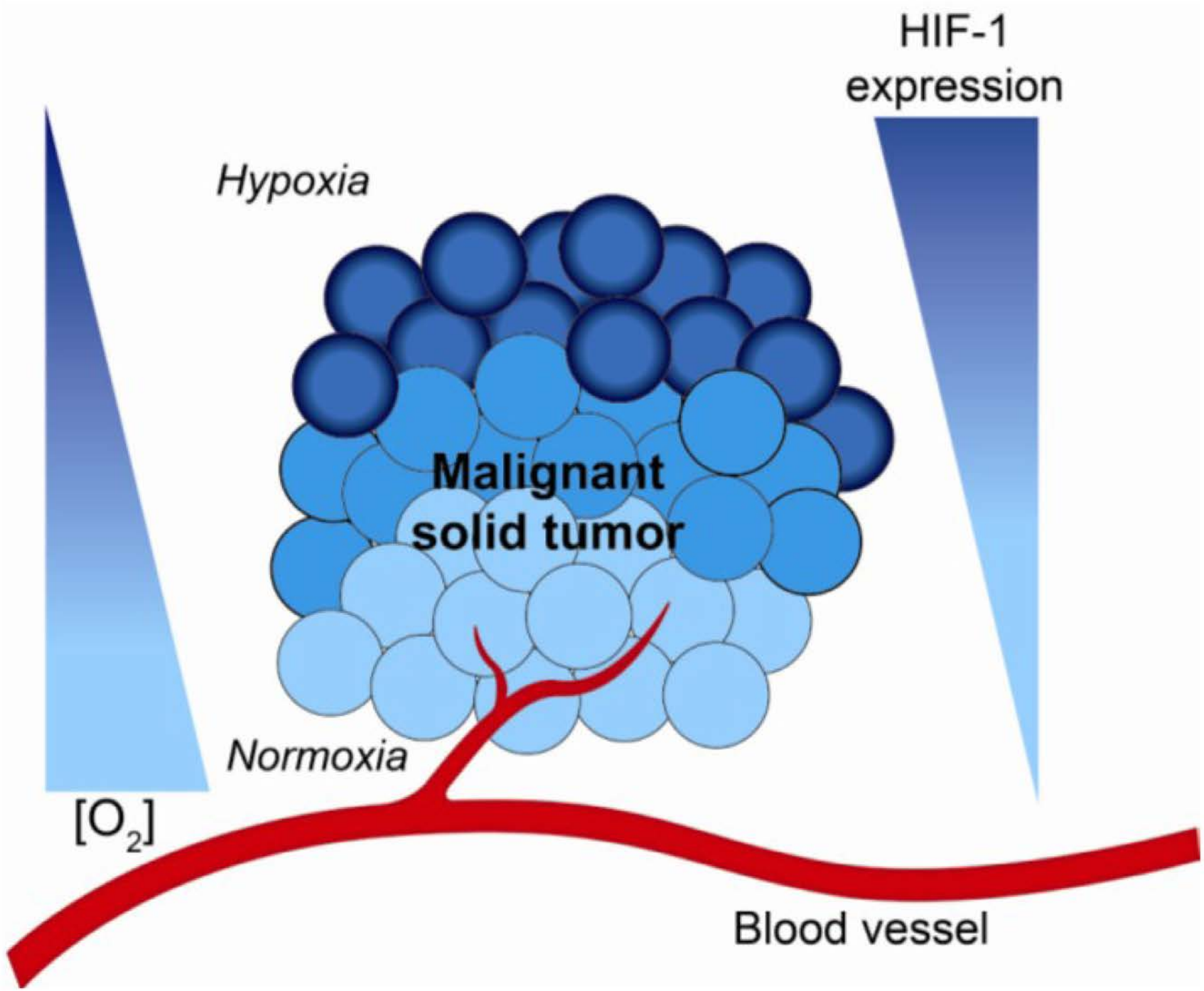
IOX2 abolishes resveratrol's effects on PC3 cell growth



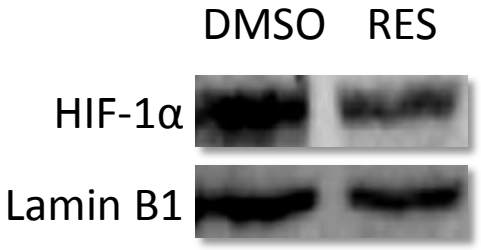


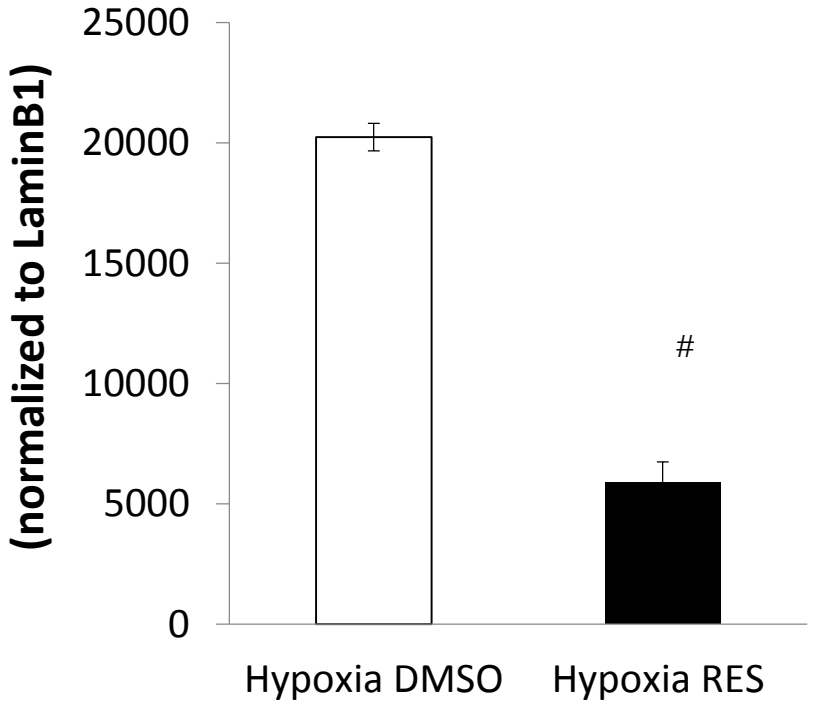
DMSO RES

Since resveratrol negatively affects HIF-1a stabilization is it particularly effective at inhibiting cancer growth in hypoxic conditions?

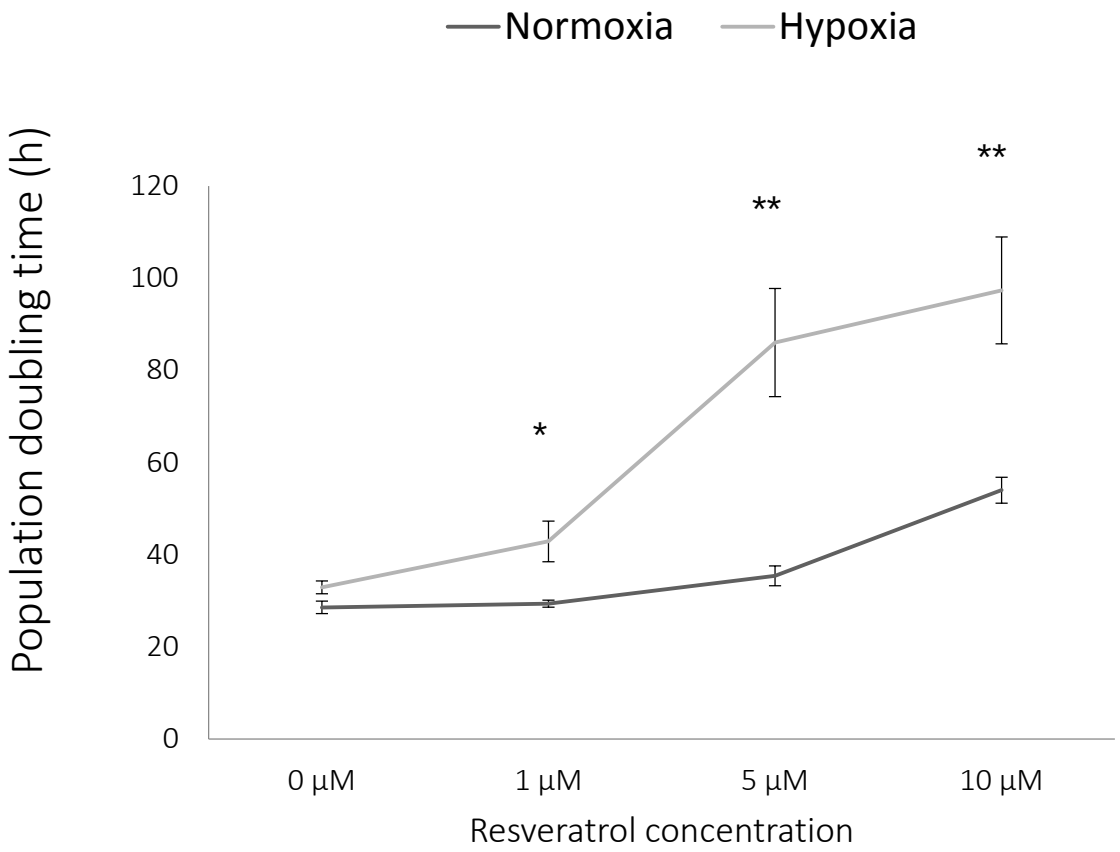

HIF-1
expression
Hypoxia
Malignant
solid tumor
Normoxia
[O2]
Blood vessel

RES prevents the stabilization of HIF-1a in hypoxia





Resveratrol's growth inhibition effect is greatly increased under hypoxic conditions

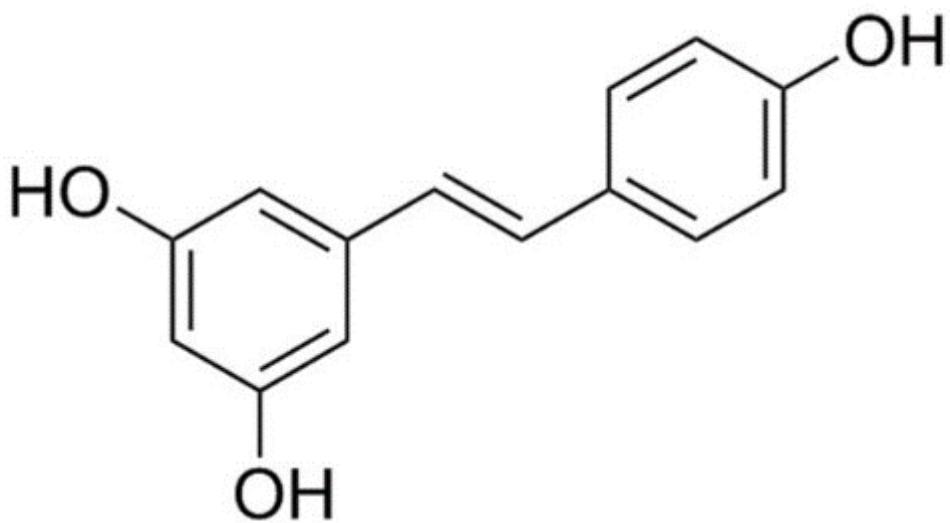


Funding


Ontario
MINISTRY OF
TRAINING, COLLEGES AND UNIVERSITIES


NSERC
CRSNG


Canada Foundation for Innovation
Fondation canadienne pour l'innovation



Resveratrol:

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Industrial partnership with Steve Murdza and Sweet & Sticky

NSERC CRSNG

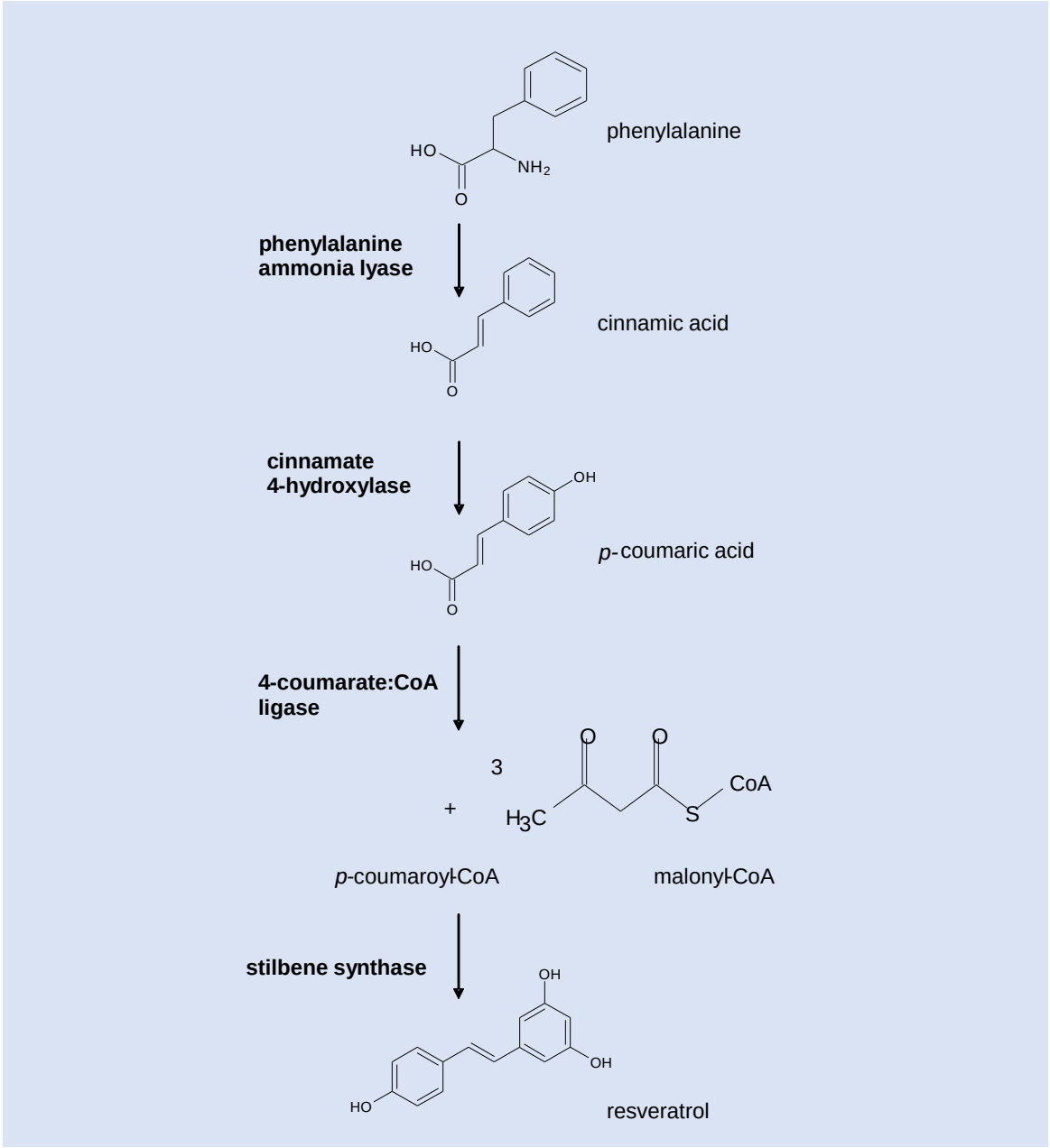
The NSERC 'Engage Grant'

Creating new research collaborations between Industry and universities

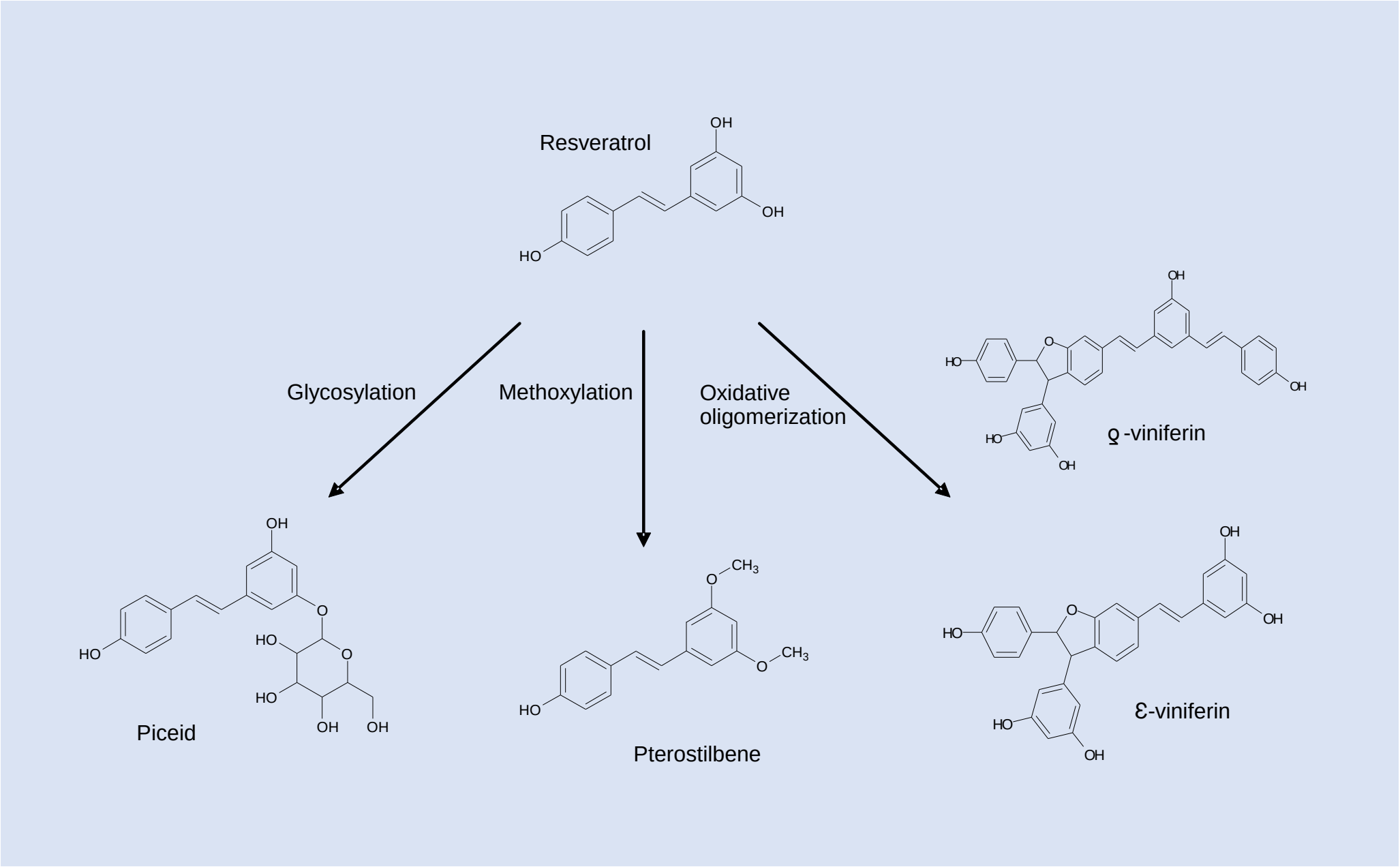
Ontario Centres of Excellence

Ontario Genomics

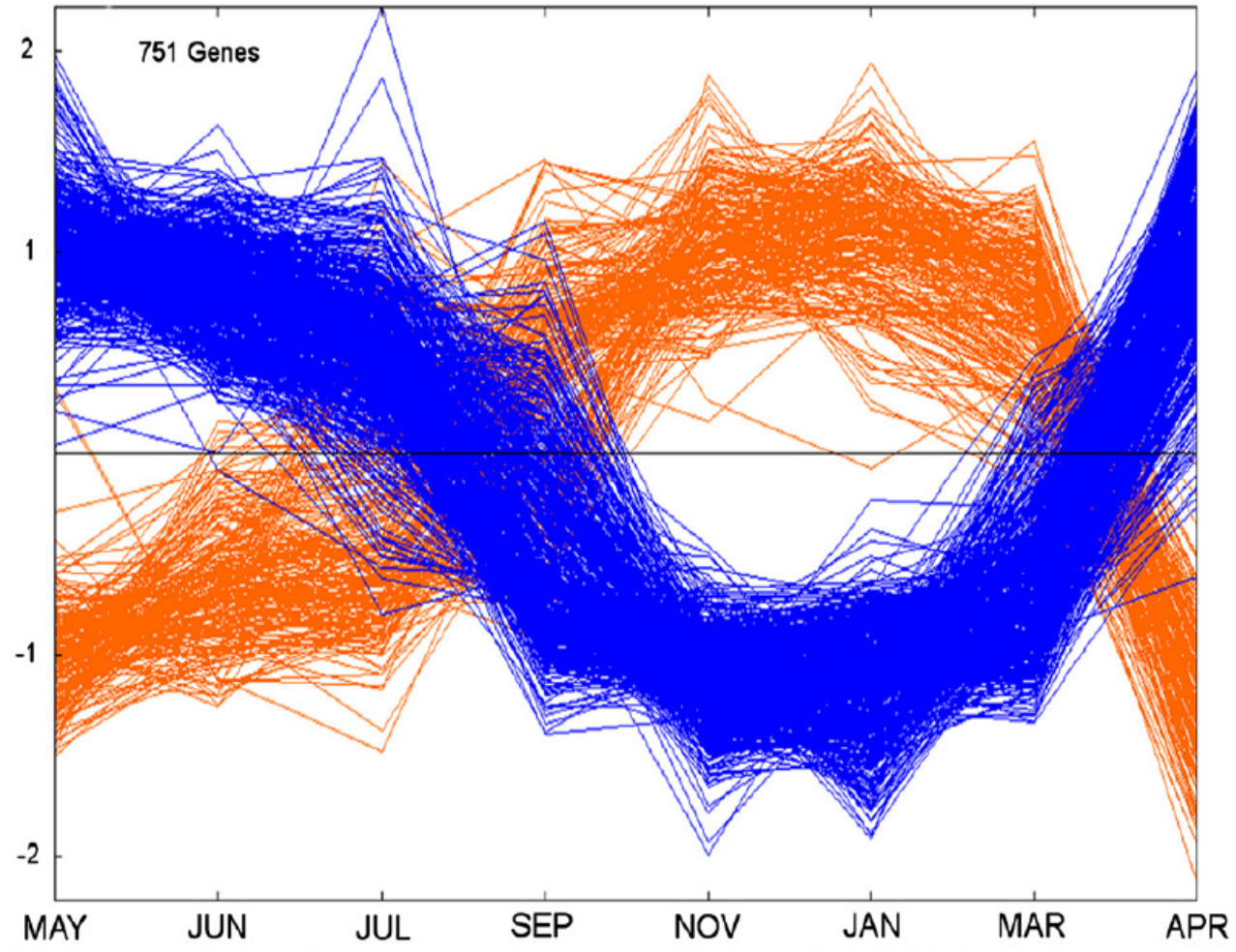
Resveratrol synthesis in *Vitis vinifera*

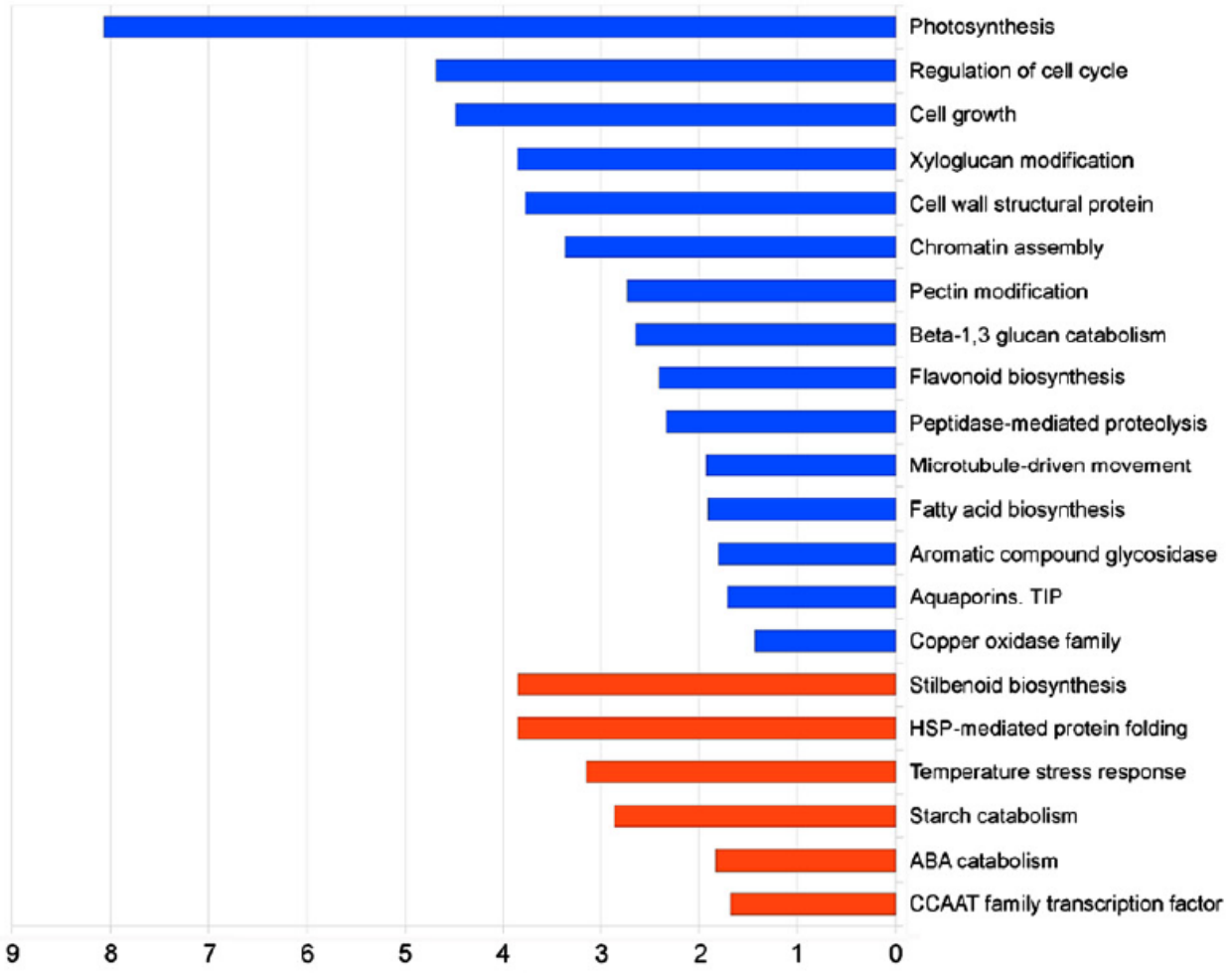


Resveratrol is a precursor to many different stilbenes



Increased stilbene synthesis gene expression in fall/winter





Díaz-Riquelme *et al. BMC Plant Biology* 2012, **12**:181

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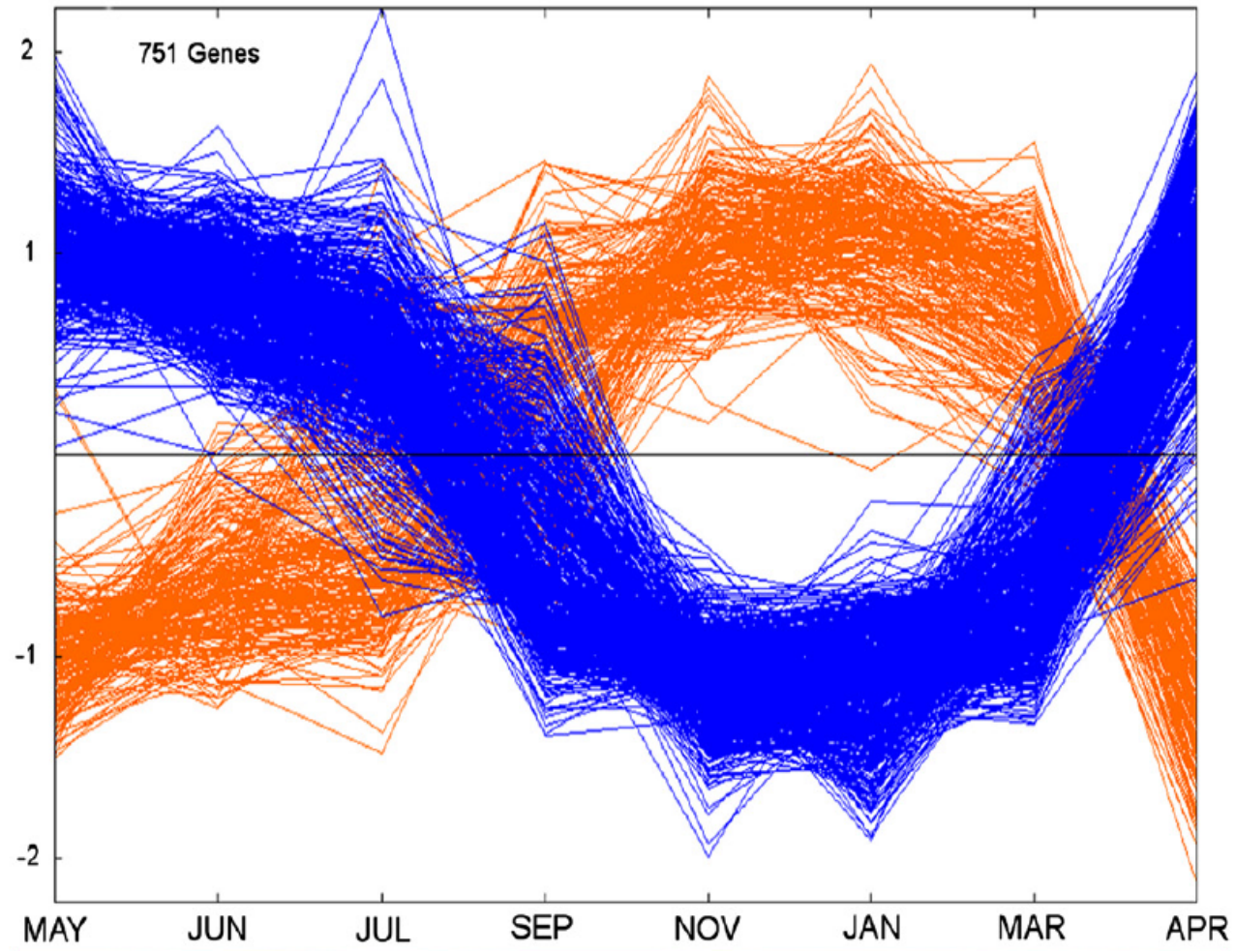
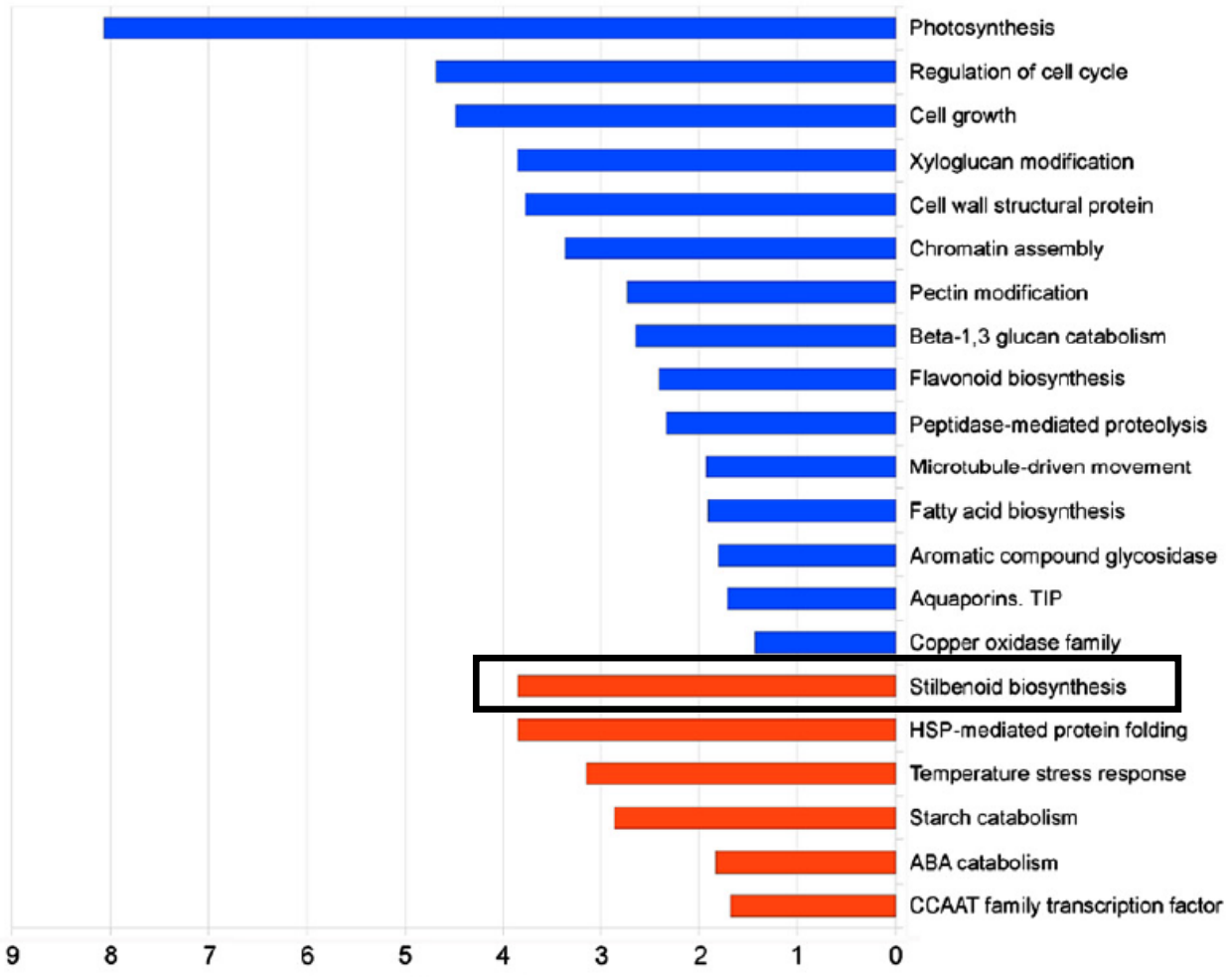
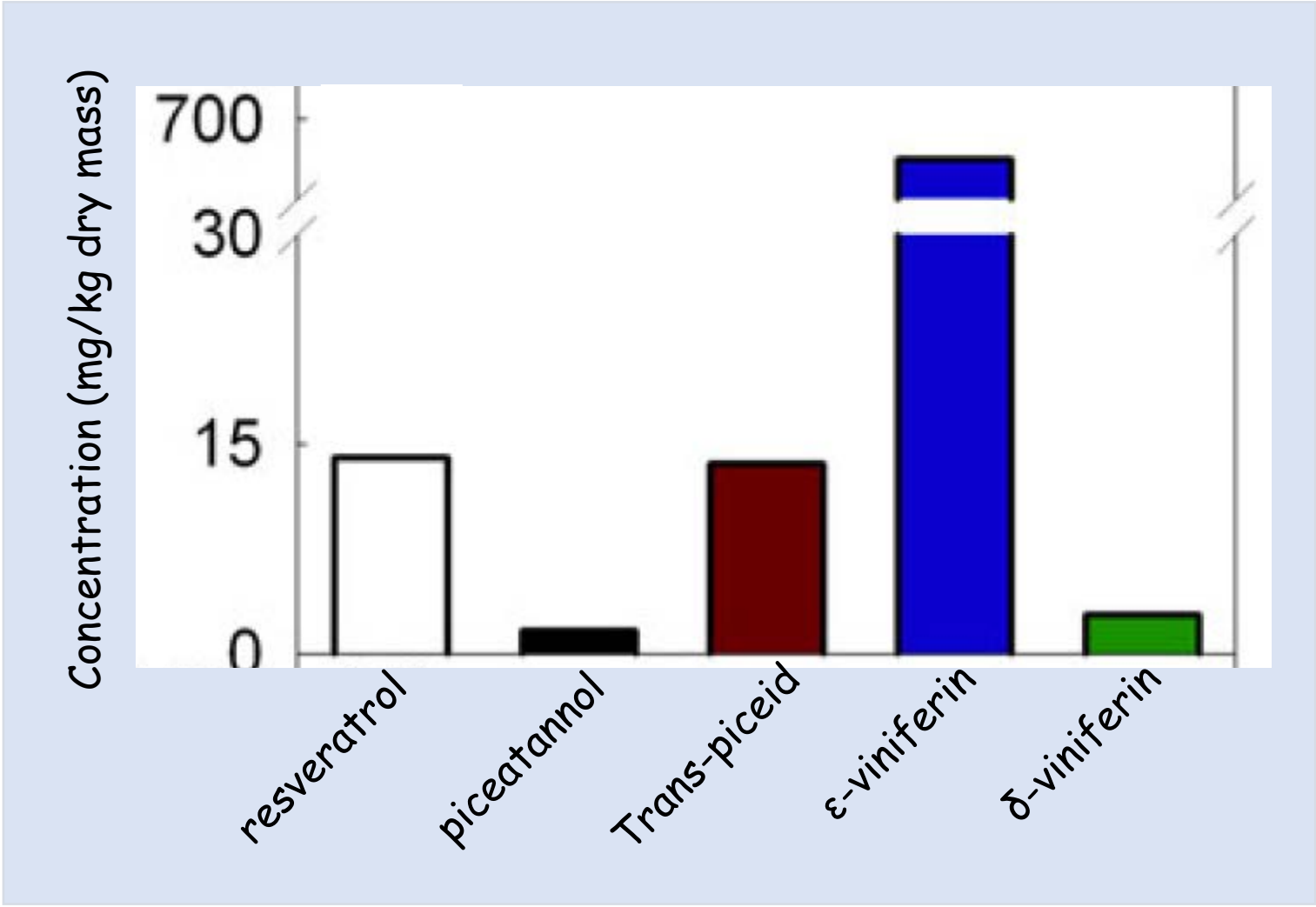




Previous collaboration between Sweet & Sticky and Vince DeLuca

- higher resveratrol levels in winter-harvested grapes used in Ice Syrup production

Grape skins contain high levels of stilbenes

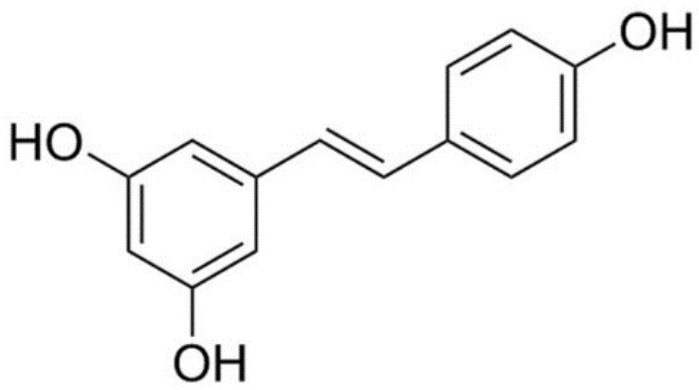


Australian Journal of Grape and Wine Research **22**, 226–231, 2016

Resveratrol is highly hydrophobic

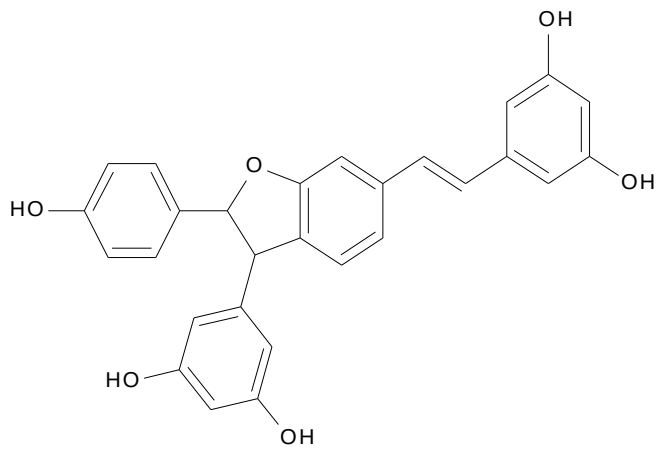
- negligible solubility in water

Solvent	Solubility of Resveratrol (g/L)
Water	0.03
Alcohol	50



Some derivatives of resveratrol are even less water soluble

Stilbene	Solubility in water (g/L)
Resveratrol	0.03
ε-viniferin	0.0001319



How to extract resveratrol and other stilbenes from the waste pomace produced during the manufacture of Ice Syrup?

Resveratrol extraction from Ice Wine grape pomace

- Process must be 'green'
  - no toxic solvents
  - product must be edible
- Process must be simple, scalable
- Resveratrol/stilbene content should be maximized
- Ideally, resultant extract should be water soluble

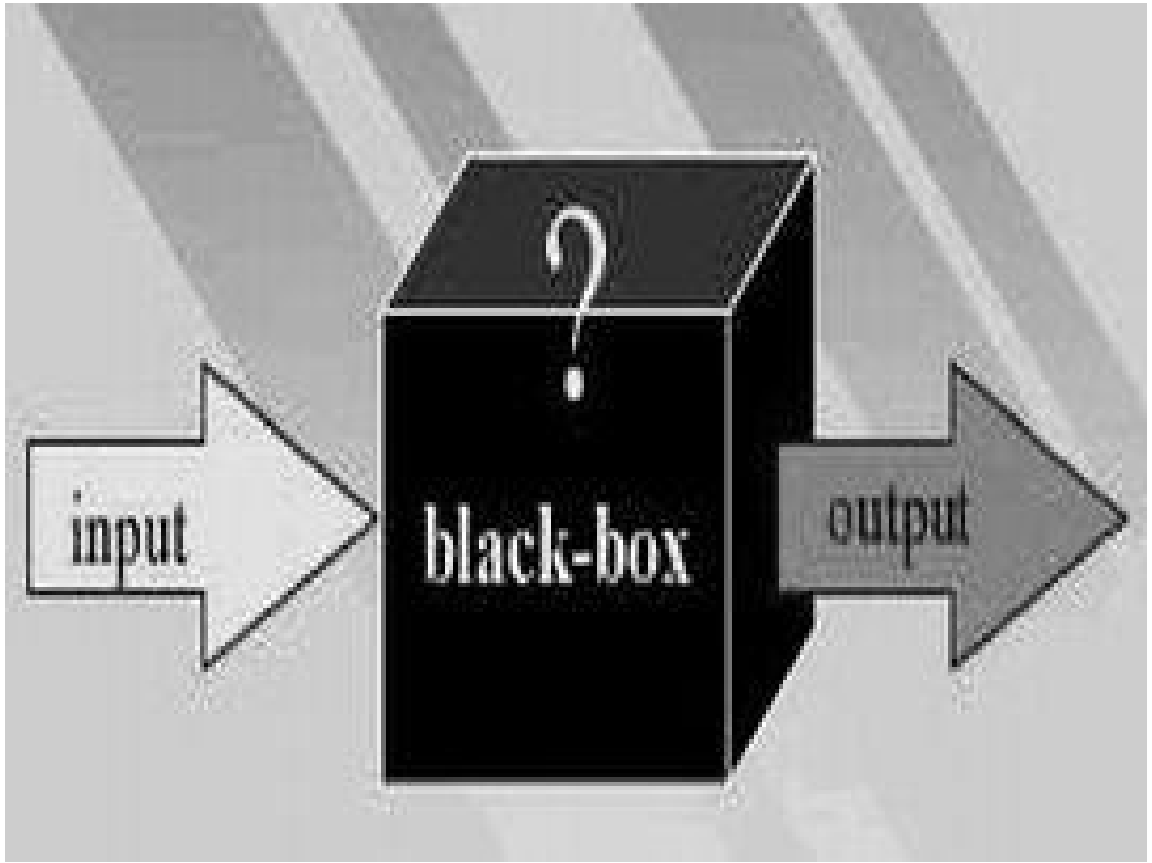
Study design:

- Two varieties of grape used in Ice Syrup production: Cabernet Franc and Vidal (Steve Murdza)

- Harvest in October, November, December, sometimes January

- Harvest years 2015, 2016, 2017

Apply the developed green extraction method to all pomace samples



Metabolomics profiling of polyphenols in all extracts

Concentration of polyphenols in extracts (µg/mg extract mass)

Samples	Treatments					
	Nov Cab Franc 2mL/g	Nov Cab Franc 2mL/g	(1)NCF 5mL/g	(2)NCF 5mL/g	(1)NCF 7mL/g	(2)NCF 7mL/g
Gallic Acid	1.15	1.04	0.46	0.52	0.21	0.21
Protocatechuic acid (3,4-Dihydroxybenzoic acid)	0.29	0.30	0.01	0.01	N/A	N/A
Catechin	3.74	3.66	1.69	1.73	1.10	1.14
Chlorogenic Acid	2.00	1.91	0.75	0.81	0.17	0.15
Vanillic Acid	0.71	0.69	0.06	0.08	0.02	0.01
Caffeic Acid	2.46	2.36	1.06	1.11	0.55	0.58
Syringic Acid	0.93	0.89	0.36	0.34	0.28	0.24
Benzoic Acid	1.30	1.27	0.49	0.51	0.21	0.21
Sinapic Acid	1.36	1.25	0.29	0.29	0.25	0.26
Rutin	0.49	0.47	0.14	0.15	N/A	N/A
Resveratrol	0.58	0.54	0.27	0.30	0.23	0.21
Quercetin	0.20	0.18	0.11	0.12	0.11	0.13
Kaempferol	0.21	0.19	0.16	0.17	0.16	0.17

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How much resveratrol is present in the pomace extracts?

- An average red wine has 1.9 ± 1.7mg/L *
  - An average glass of wine is about 175ml
    - Therefore, average glass of wine has ~0.33mg resveratrol

- The best extracts have ~0.6mg/g
    - Therefore, ~0.5g of the extract is equivalent to a glass of wine

- The extracts have no alcohol and contain a variety of 'good' polyphenols

* Sabine Weiskirchen and Ralf Weiskirchen *Adv Nutr* 2016;7:706–18;

If the extracts are added to Ice Syrup (they are water soluble), what concentration of resveratrol could be achieved?

Up to 1.5g of extract per 100ml Ice Syrup

Therefore, almost 1mg of resveratrol per 100ml Ice Syrup

Compare to 0.19mg resveratrol per 100ml of an average red wine

Relative levels of some other polyphenols in the extracts versus red wine

Compound	Level in Extract (mg/g)	Amount in glass of red wine (mg/175 mls) *
Resveratrol	0.56	0.33
Kaempferol	0.20	0.2-2
Catechin	3.70	100-150
Quercitin	0.19	5-20

*Granato et al 2011; Food Chemistry

Conclusions

(1) Resveratrol has some health benefits that have been well studied. These include anti-cancer activities.

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(4) An edible extract can be produced from the pomace of Cabernet Franc and Vidal grapes harvested in October through December (maybe January). The CF extracts have resveratrol levels comparable to red wines but without the alcohol. More characterization is needed.

Acknowledgements:

- Elaine Corbett and Dennis McCormick – Ontario Genomics
- Rupasri Mandal and Jen Reid – TMIC (The Metabolomics Innovation Centre)
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