

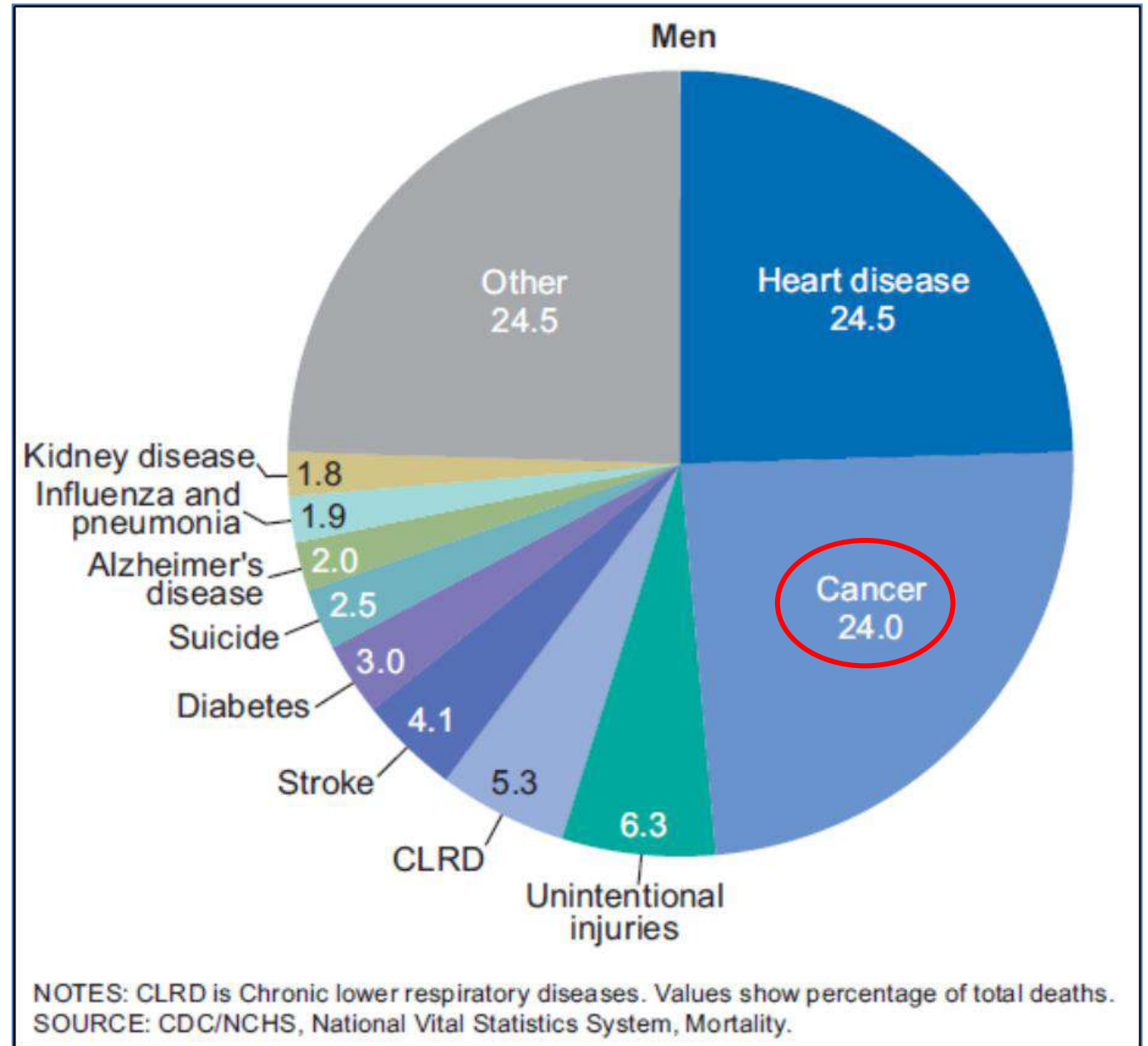
HOW VASCULAR CELL ADHESION GENE IS DOWNREGULATED BY COLOMBIAN PROPOLIS IN TUMOR CELLS

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Cancer is a common and growing disease

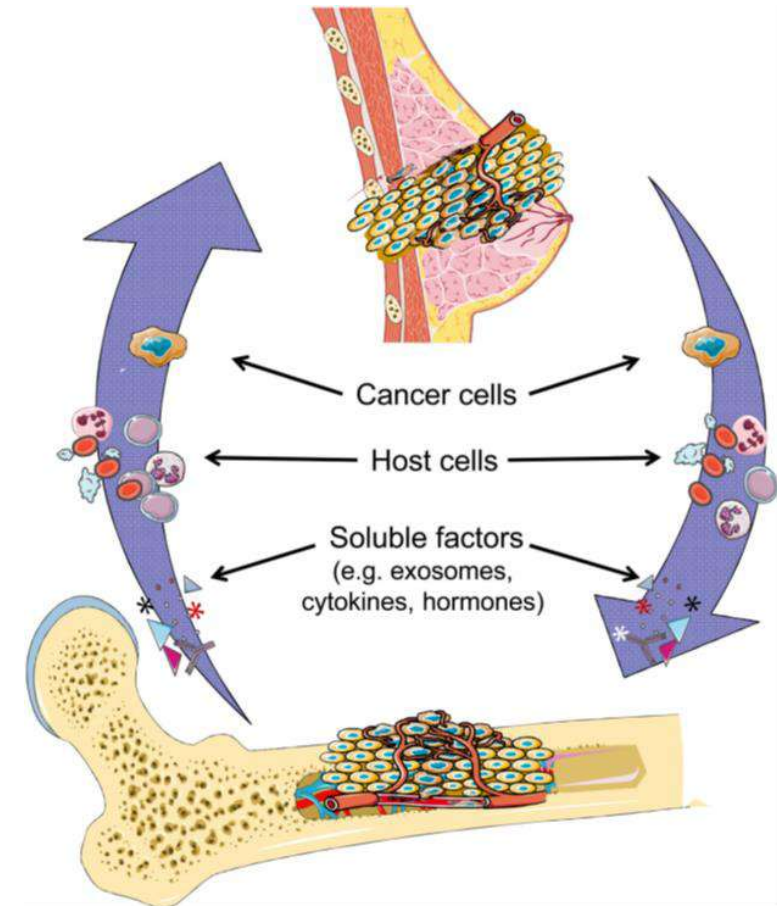
Incidence

There are more than 277 different types of cancer.



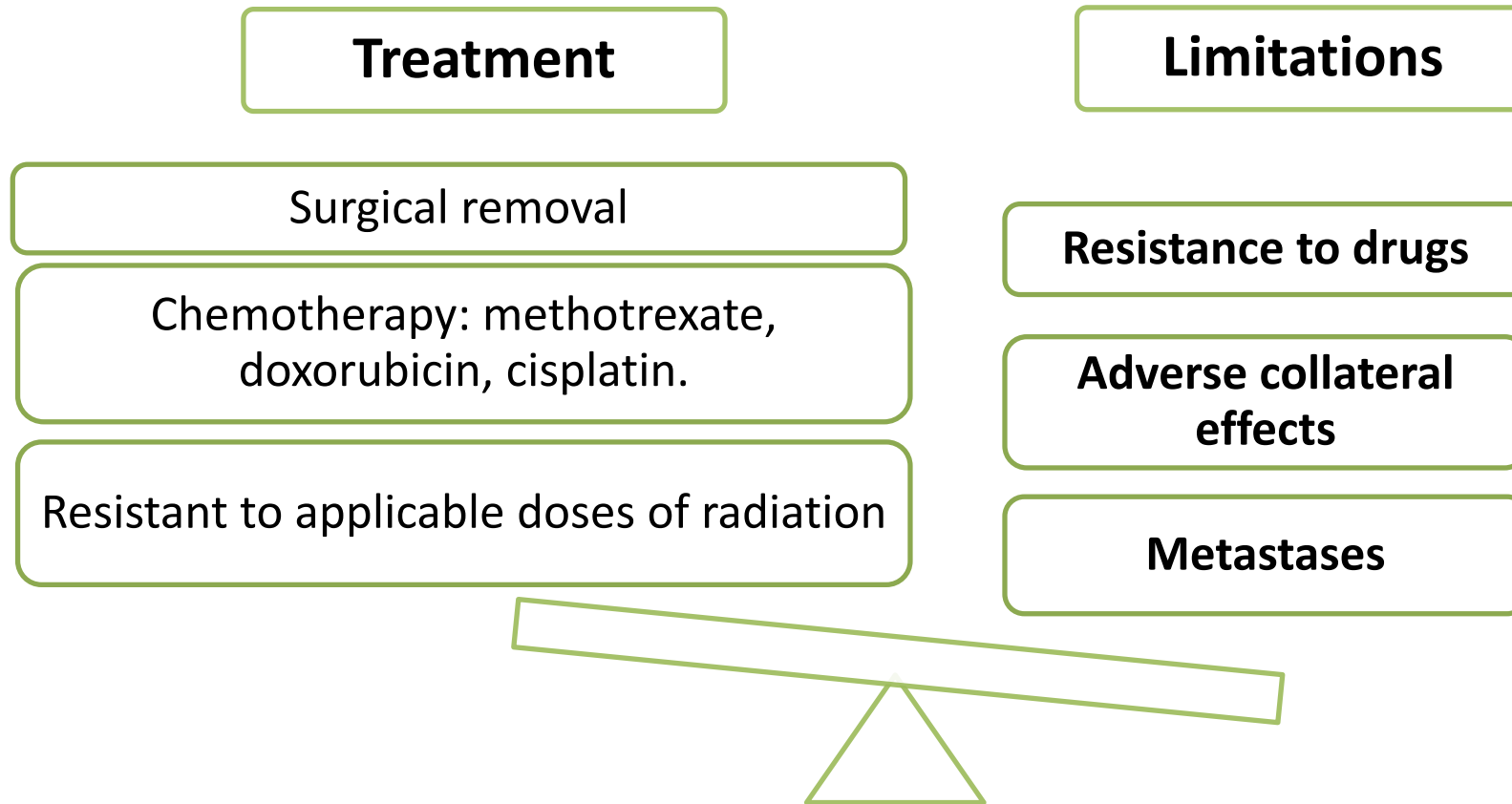
Osteosarcoma (OSA) is one bone cancer type

- Third highest cause of cancer in adolescents
- Locally aggressive
- Early systemic metastases



Pienta et al., 2013

Conventional treatments show many negative effects



*Luetke et al. 2014; Ando et al, 2012; **Morello** et al, 2011.*

Lozano, et al 2012; Holmgren et al, 2016, Zhang et al 2017.

Propolis exhibits a potential alternative treatment

PROPOLIS: Biological and pharmacological properties

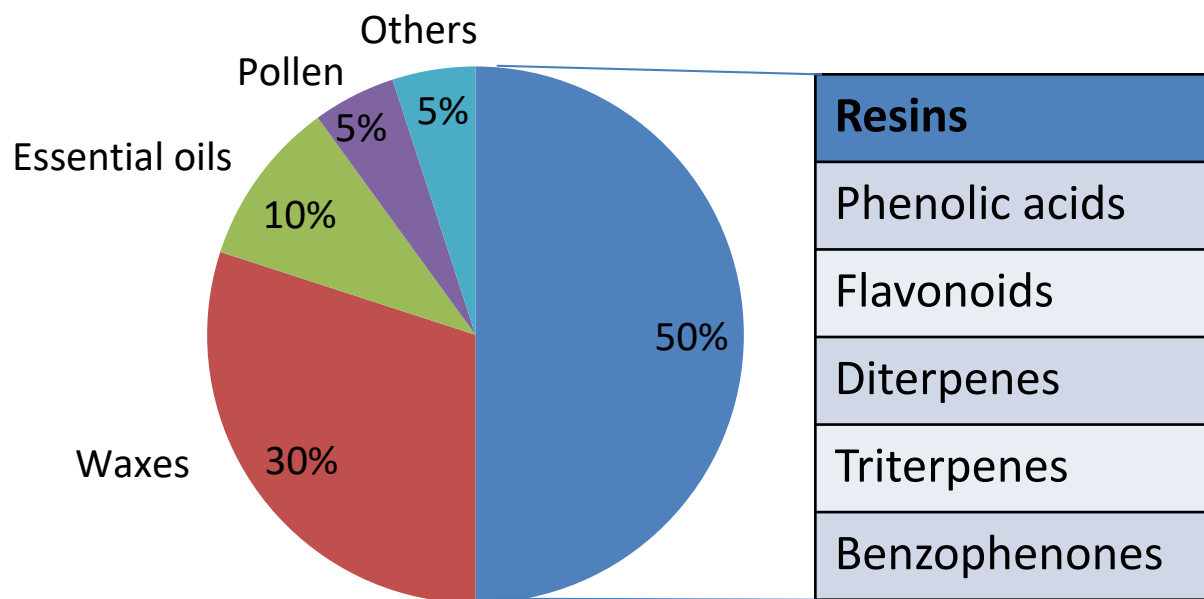
- Immunomodulatory
- Antitumor
- Anti-inflammatory



Kumazawa S, et al 2003.

Sforcin & Bankova, 2011.

The composition of propolis determines its effects



Gómez-Caravaca et al, 2006

CAPE *Natarajan, et al, 1996.*

Artepilin C, baccharin, drupanin
Kumazaki et al., 2014.

Naringenin *Park et al., 2008.*

Chrysin *Rashid et al., 2014.*

Kaempferol *Rajendran P et al., 2014.*

Nemorosone *Diaz-Carballo et al., 2008.*

The activity of propolis is a consequence of the synergism between its components *Sforcin, 2010; Huang et al., 2014.*

Composition of propolis is associated with geographical and botanical origin

Colombia is known for its great climatic diversity, including: deserts, tropical rainforests, savannas prairies and mountain ranges.

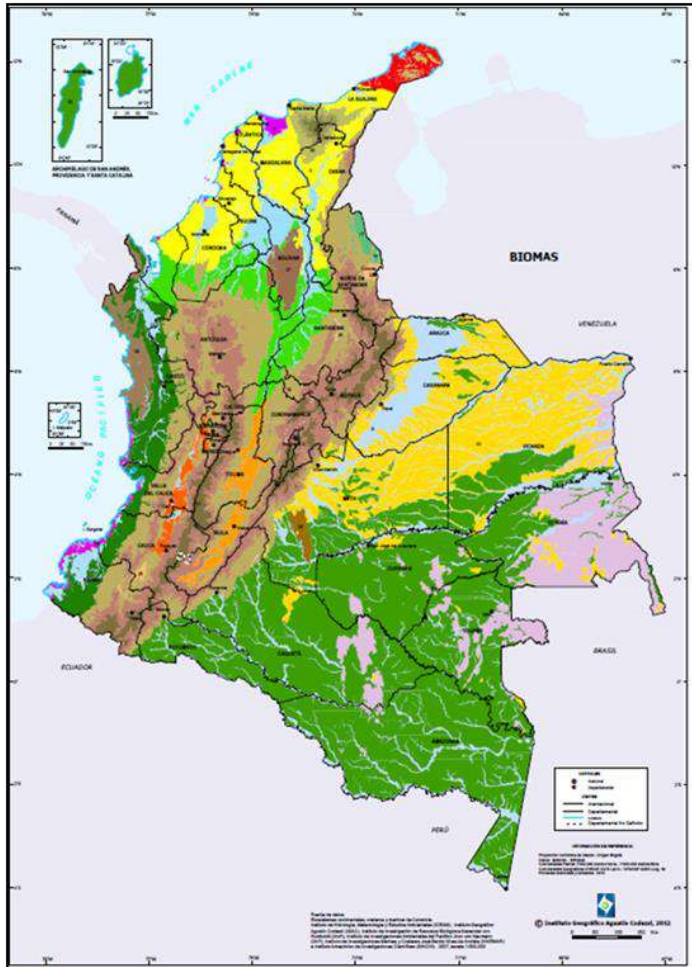
The Warm Zone
1.000 MASL.

The Temperate Zone
1.000 and 2.000 MASL.

The Cold Zone
2.000 and 3.000 MASL.

The Paramo Zone
3.000 to 4.000 MASL.

Propolis production
in Colombia



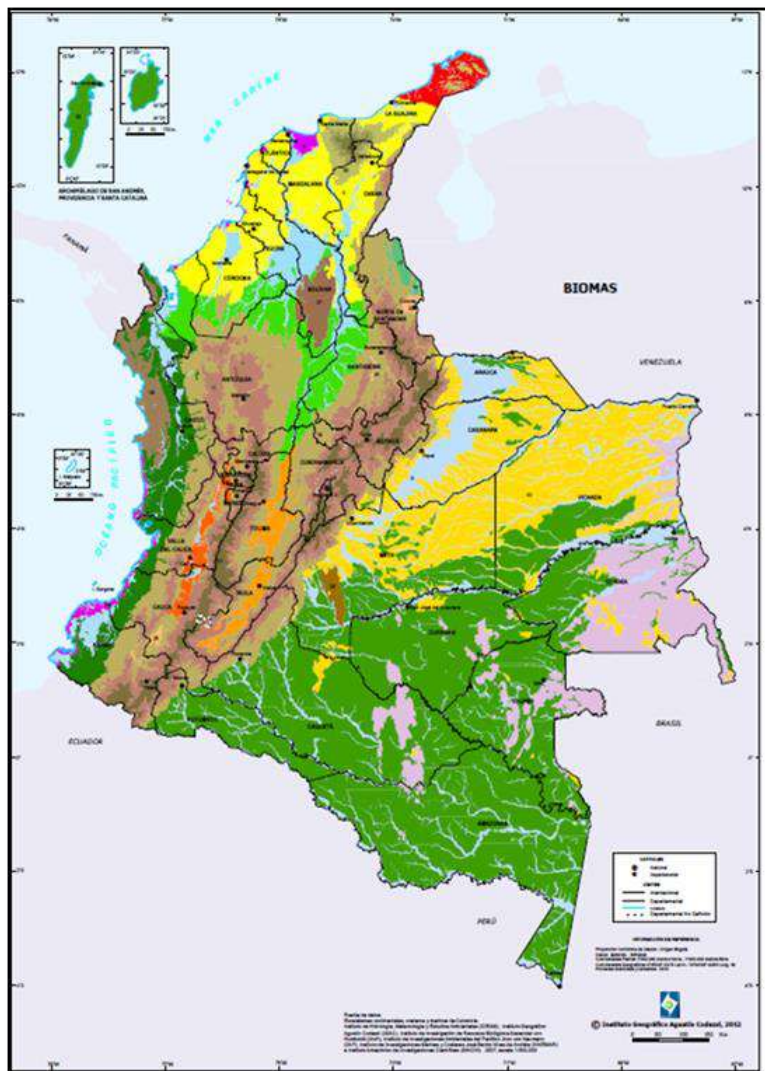
Colombian propolis can be found in several regions

Samples used in the study were chemically characterized:

Tropical zones in Colombia	MASL	Sample name
Bogota	2640	Usm
Meta	365	Met
Cauca, Silvia	2450	Sil
Cauca, Cajibío	1750	Caj



Composition of Colombian propolis samples



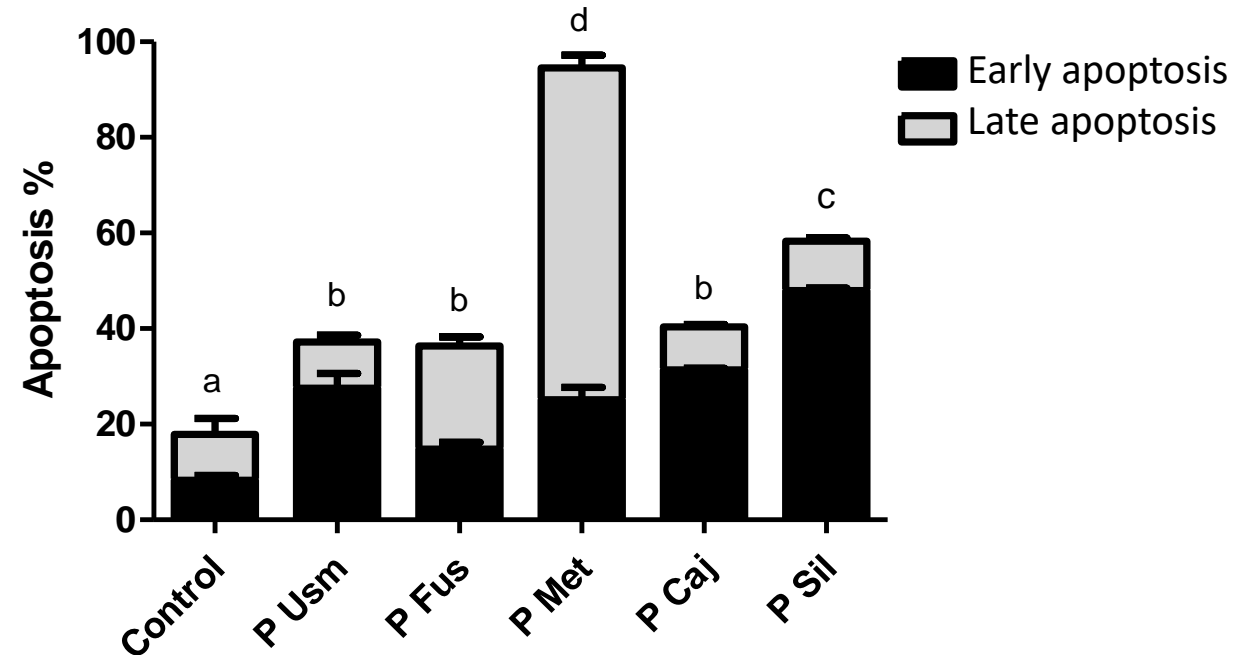
Diterpenes	Triterpenes	Benzophenones	Flavonoids
Levopimaric acid	Lanosterol	Nemorosone	Kaempferol
Agatadiol	β-Amirine	Propolone A	Dihydroxi-metoxiflavone
Diterpenes (unidentified)	Lupeol	Propolone A (isomer)	
	Cicloartenol	Nemorosone (isomer)	
	Triterpenes (unidentified)	Benzophenones (unidentified)	

Pardo-Mora et al., 2019.

Previous studies suggest that Colombian propolis can lead to the discovery of new anti-tumor drugs

Cytotoxic effect on canine OSA cells:

Apoptosis, mitochondrial membrane alteration, and suppression of cell invasion

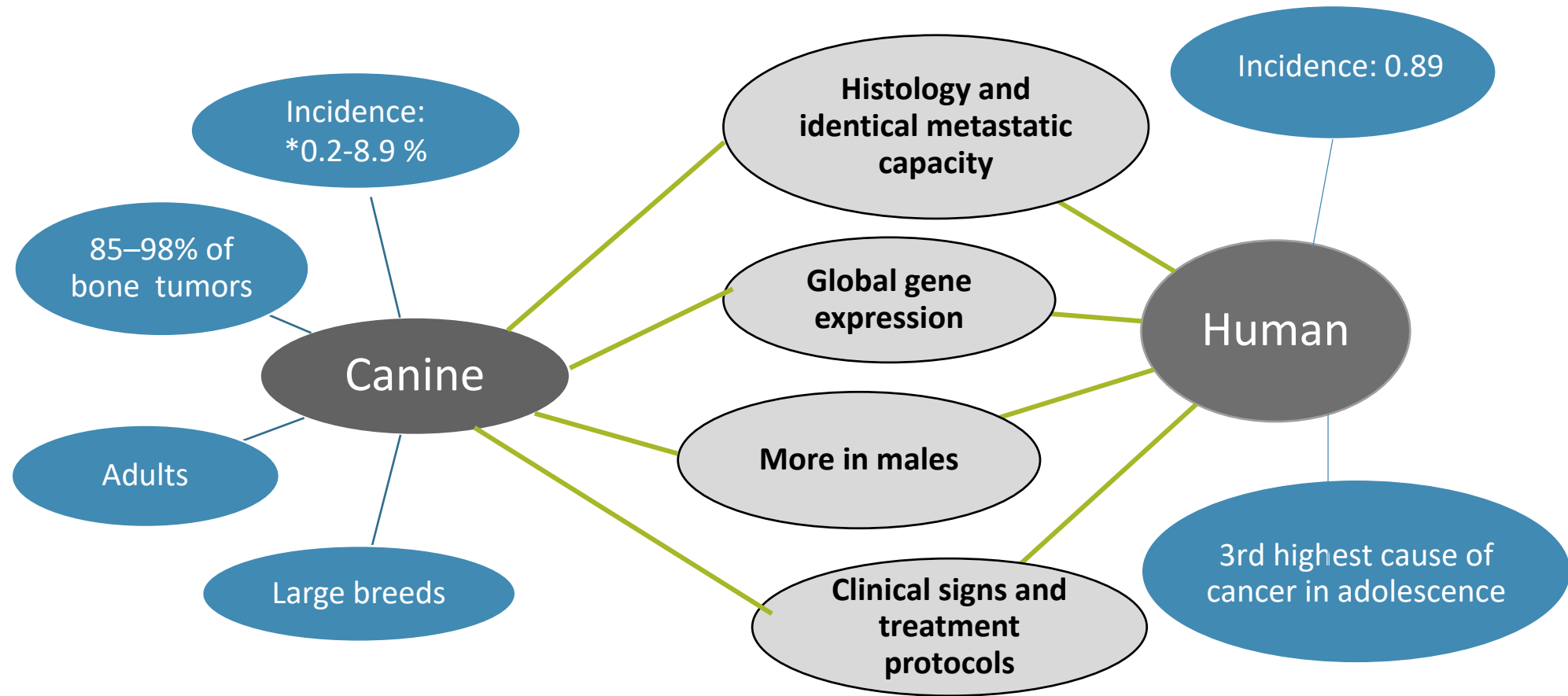


Objectives of the research

To evaluate the cytotoxic effect and the gene expression profile of canine osteosarcoma (OSA) cells treated with Colombian propolis extracts.

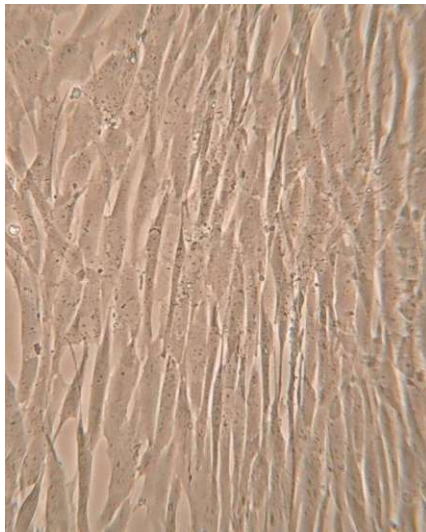
To identify differentially expressed genes, to understand which pathways could be involved in their cytotoxicity.

Why does canine osteosarcoma help us to understand human OSA?

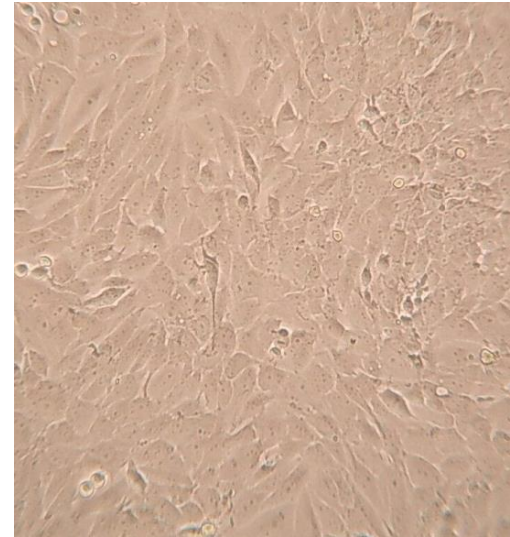


Methodology: cell culture

Primary culture
Fibroblast



Canine Osteosarcoma Cell
Line (OSCA-8) (Kerafast[®] Inc.)

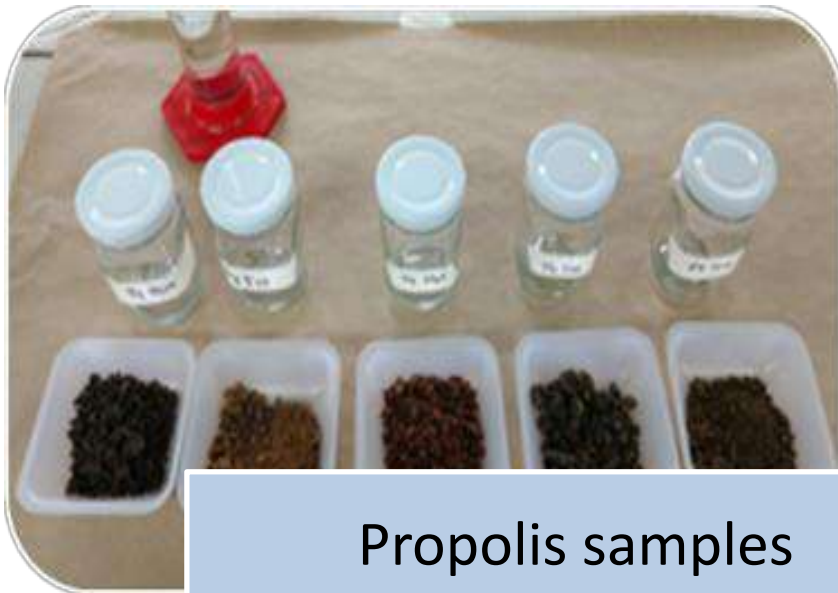


Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% fetal calf serum.
All cells were maintained at 37°C in a humidified 5% CO₂ atmosphere.

Samples obtained

Four samples were collected in Colombia: **Usm**, **Met**, **Sil** and **Caj**.

Samples were ground; propolis were dissolved in 70% **ethanol**, stored in the absence of bright light, under moderate shaking, at room temperature.



Propolis samples

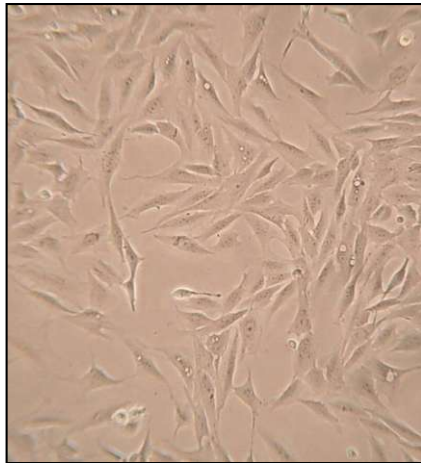


Ethanolic extract

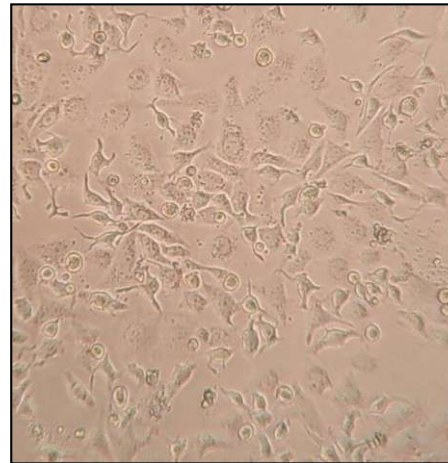
LDH assay: Cytotoxicity

Lactate dehydrogenase release in the culture medium by rupture in the cell membrane.

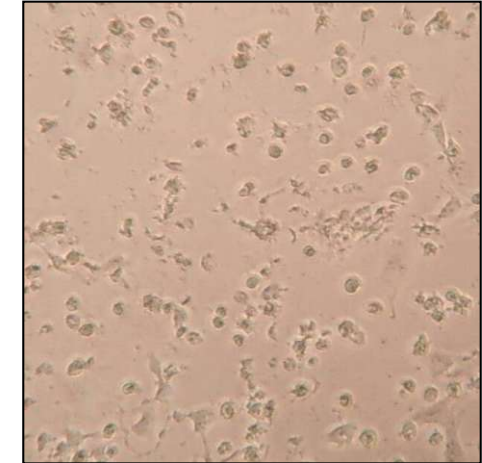
Propolis	Usm, Met, Caj and Sil
Concentration	10, 25, 50 and 100µg/ml
Incubation	48 and 72 h
Controls	Doxorubicin



Normal cells OSCA-8: 0 h



Cytotoxic effect
OSCA-8: 48 h



Cytotoxic effect OSCA-8:
72 h

Technique used to evaluate gene expression

Microarray

The Canine Gene 1.0 ST array
(Affymetrix)

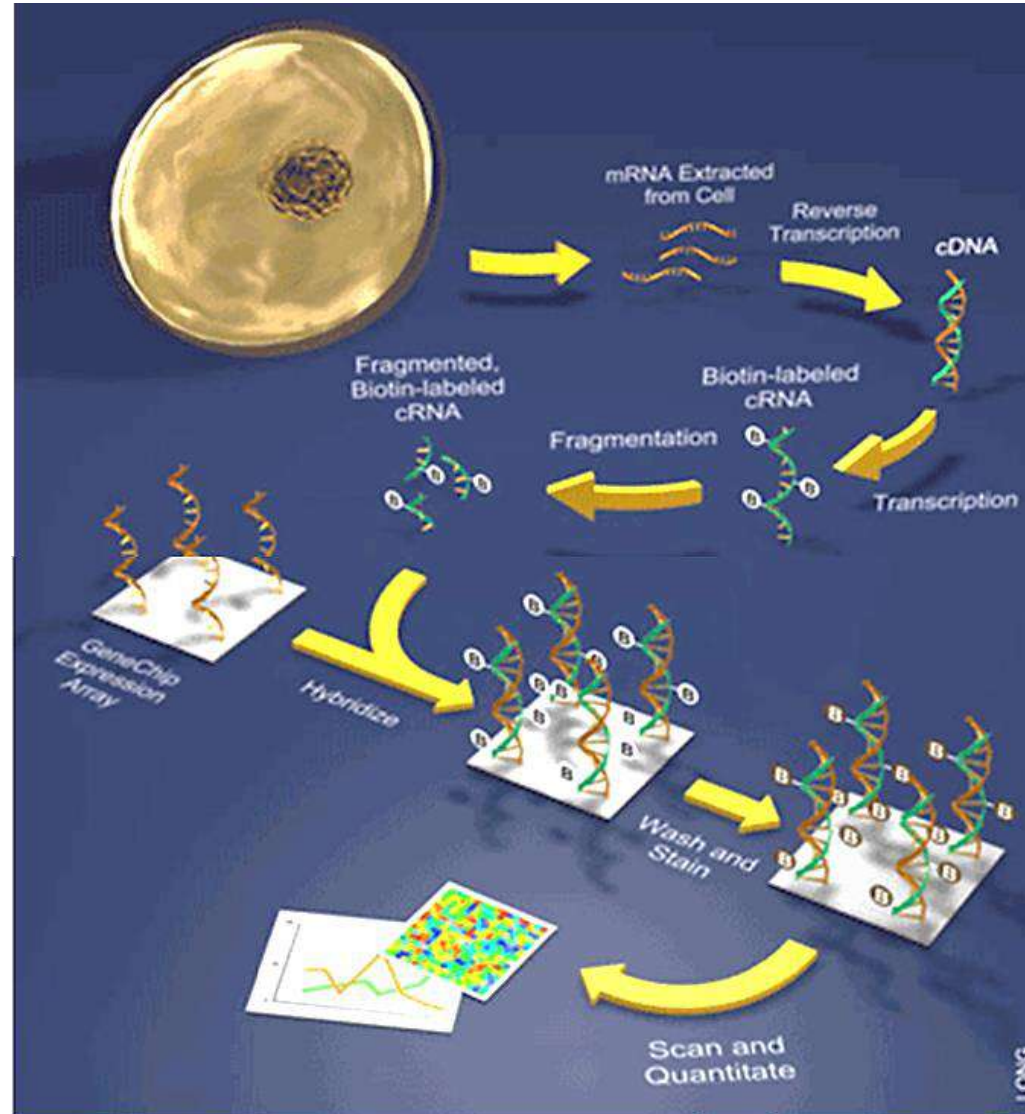


Statistical analysis

ANOVA, the Significance Analysis of
Microarrays (SAM), the false
discovery rate (FDR)

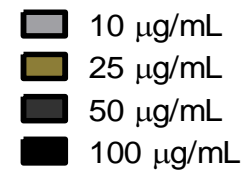
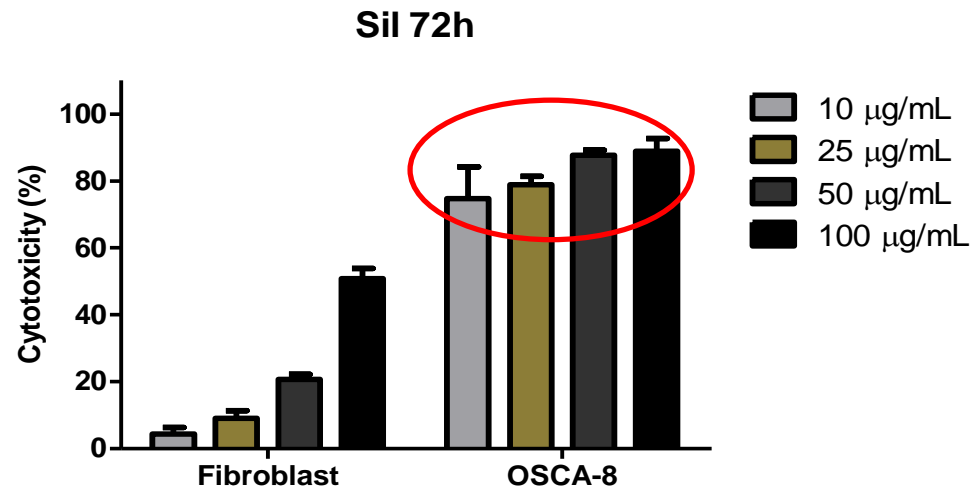
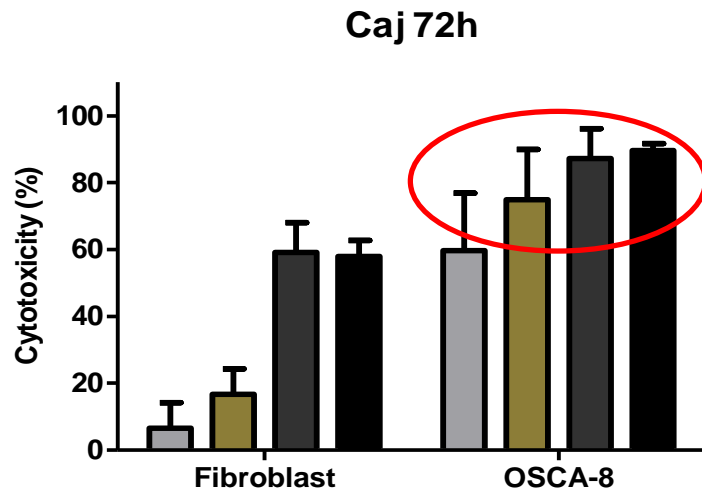
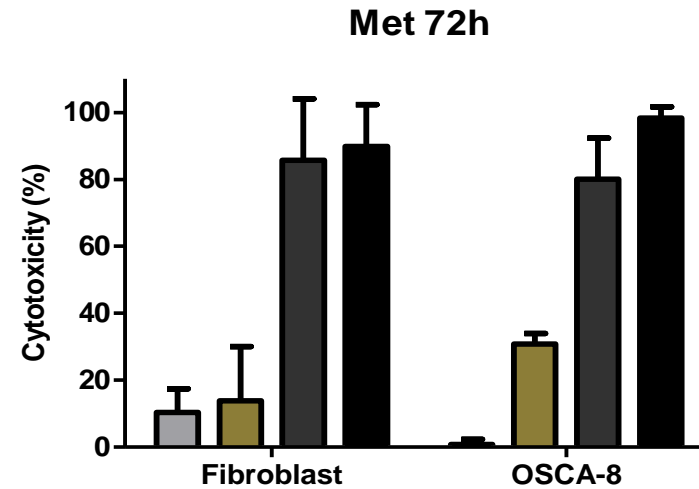
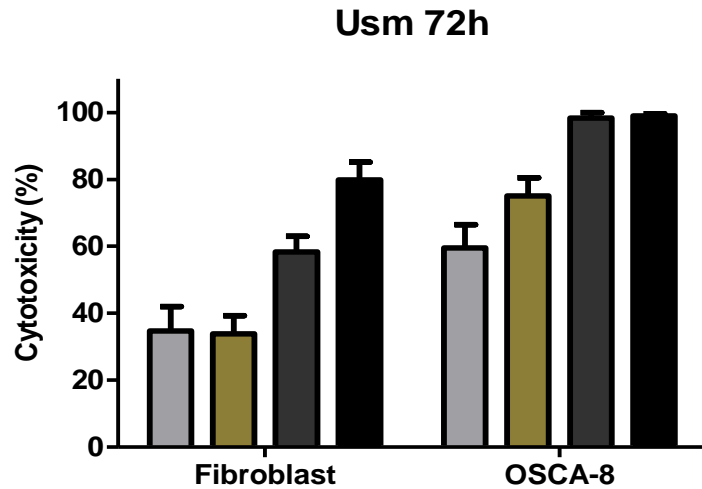


The microarray results were
validated by quantitative real time
PCR (qPCR).



Evaluation of the
differential
expression profile
of genes in
canine OSA cells
exposed to
propolis.

RESULTS: cytotoxic effect per region



The LDH assay results showed a cytotoxic effect on canine OSA cells.

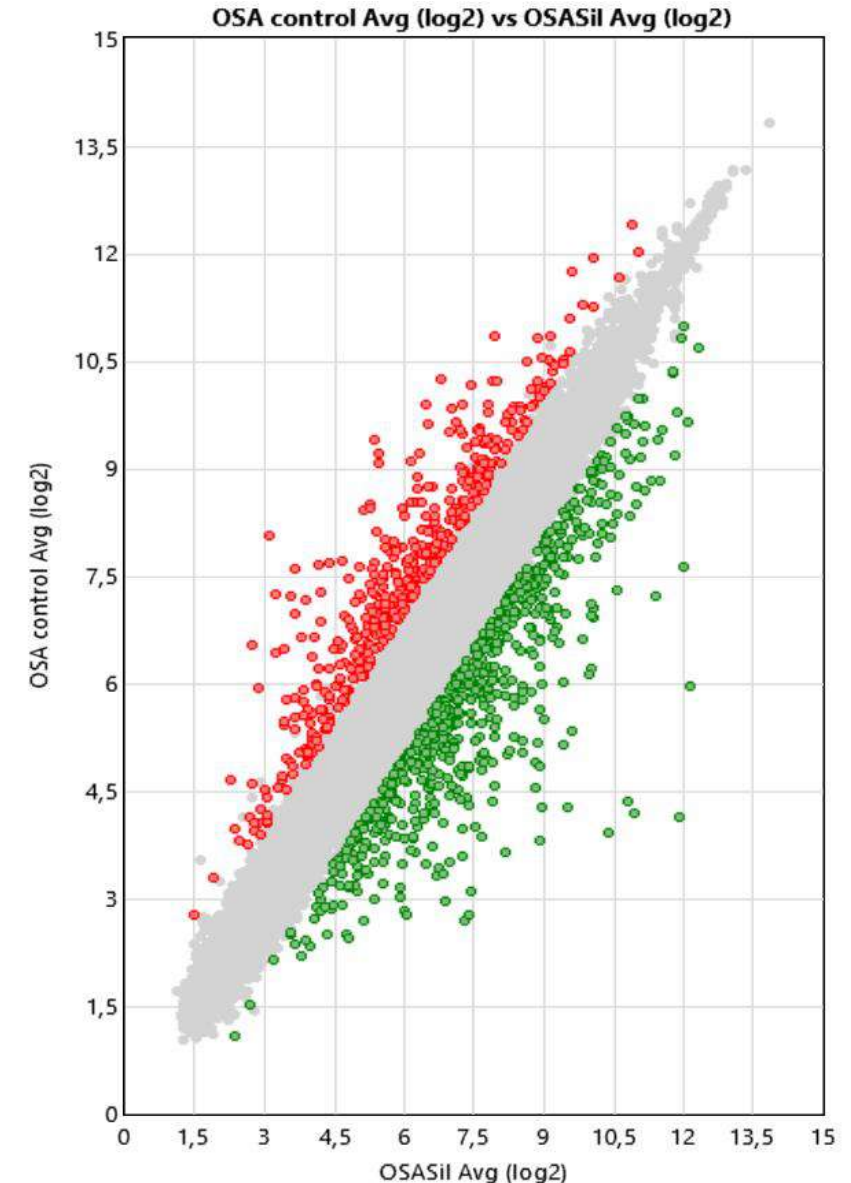
After 72 h of treatment with propolis, the CC₅₀ in OSCA-8 cells were lower than 30 µg/mL.

Selectivity index: >1

Summary of changes in gene expression

Differential expression of genes in cells exposed to propolis extracts

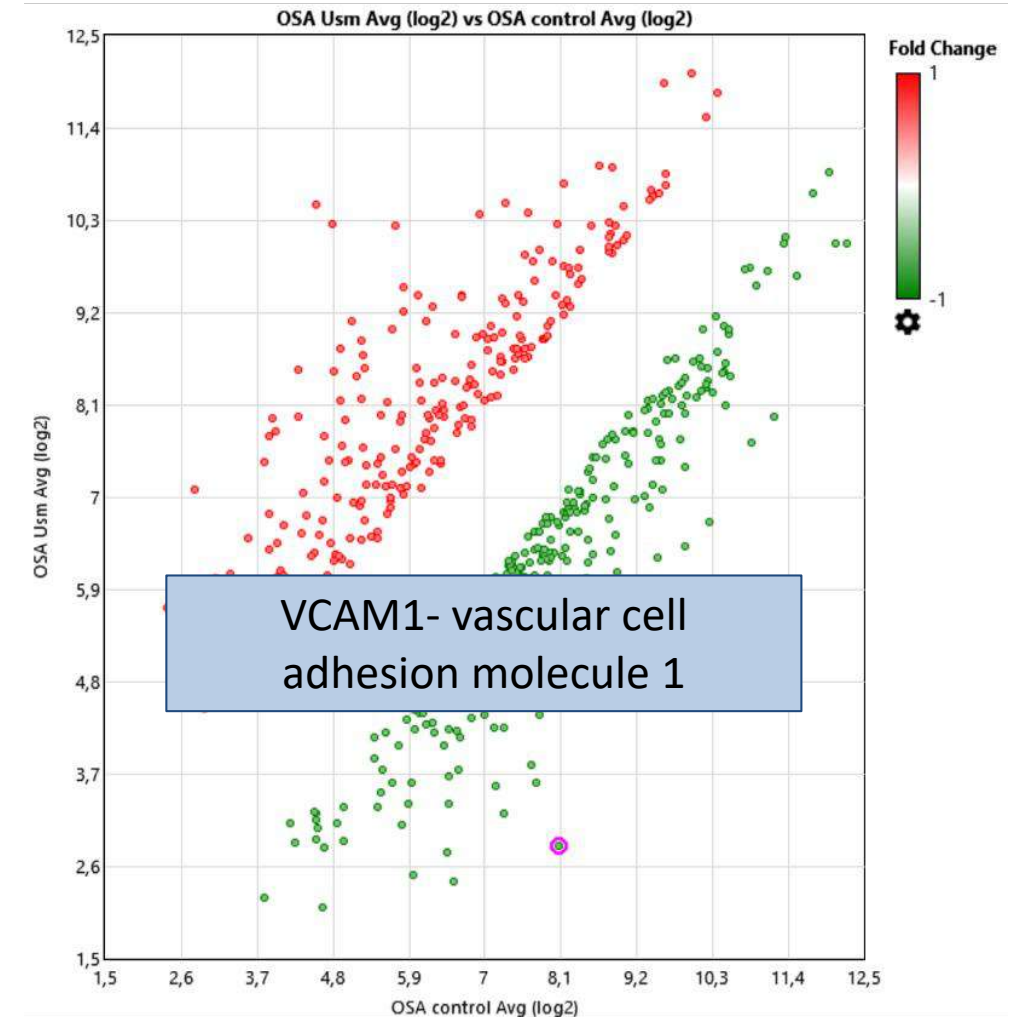
Comparación	Δ	FDR	Up	Low	Total
OSA-USm	0.75	0.0019	164	178	342
OSA-Sil	0.825	0.0018	233	110	343
OSA-Met	1.43	0.002	185	153	338
OSA-Caj	0.7	0.0016	291	103	394
OSA-Dox	1	0.0015	164	171	335



RESULTS: VCAM1

Genes differentially expressed in at least two treatments including Dox treatment.

Gene Name	Doxo	Sil	Caj	Usm	Met
VCAM1 – vascular cell adhesion molecule 1	Down	Down	Down	Down	Down
ST6GAL2 – ST6 beta-galactoside alpha-2,6-sialyltransferase 2	Down	Down	Down		
TOX – thymocyte selection associated high mobility group box	Down	Down			
ARHGAP28 – Rho GTPase activating protein 28	Down	Down			
CXCL8 – C-X-C motif chemokine ligand 8	Up		Up	Up	
ABCB1 – ATP binding cassette subfamily B member 1	Up		Up	Up	Up
PTGS2 – prostaglandin-endoperoxide synthase 2	Up		Up	Up	
SERPINB2 – serpin family B member 2	Up		Up	Up	

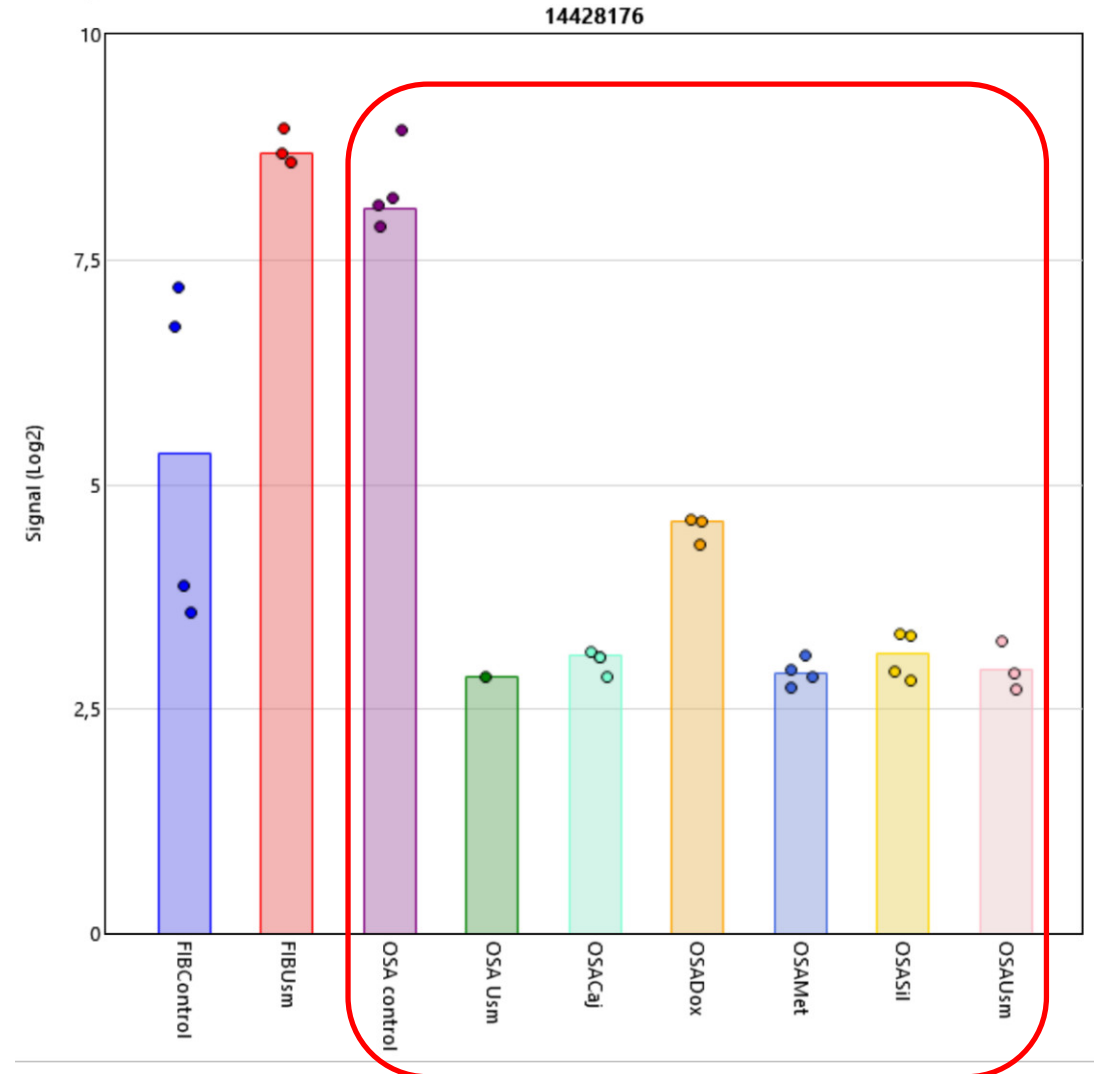


RESULTS: large effect on expression of VCAM1

VCAM1 is involved in a wide array of biological processes such as hemostasis, the immune response, and inflammation.

Gene Symbol: VCAM1

Description: vascular cell adhesion molecule 1



Why is VCAM1 so important in cancer?

VCAM-1 is commonly highly overexpressed.

**In Breast
cancer cells**

Wang et al., 2014.

**In metastatic breast cancer
cells to the lungs, lung
cancers, colorectal cancer.**

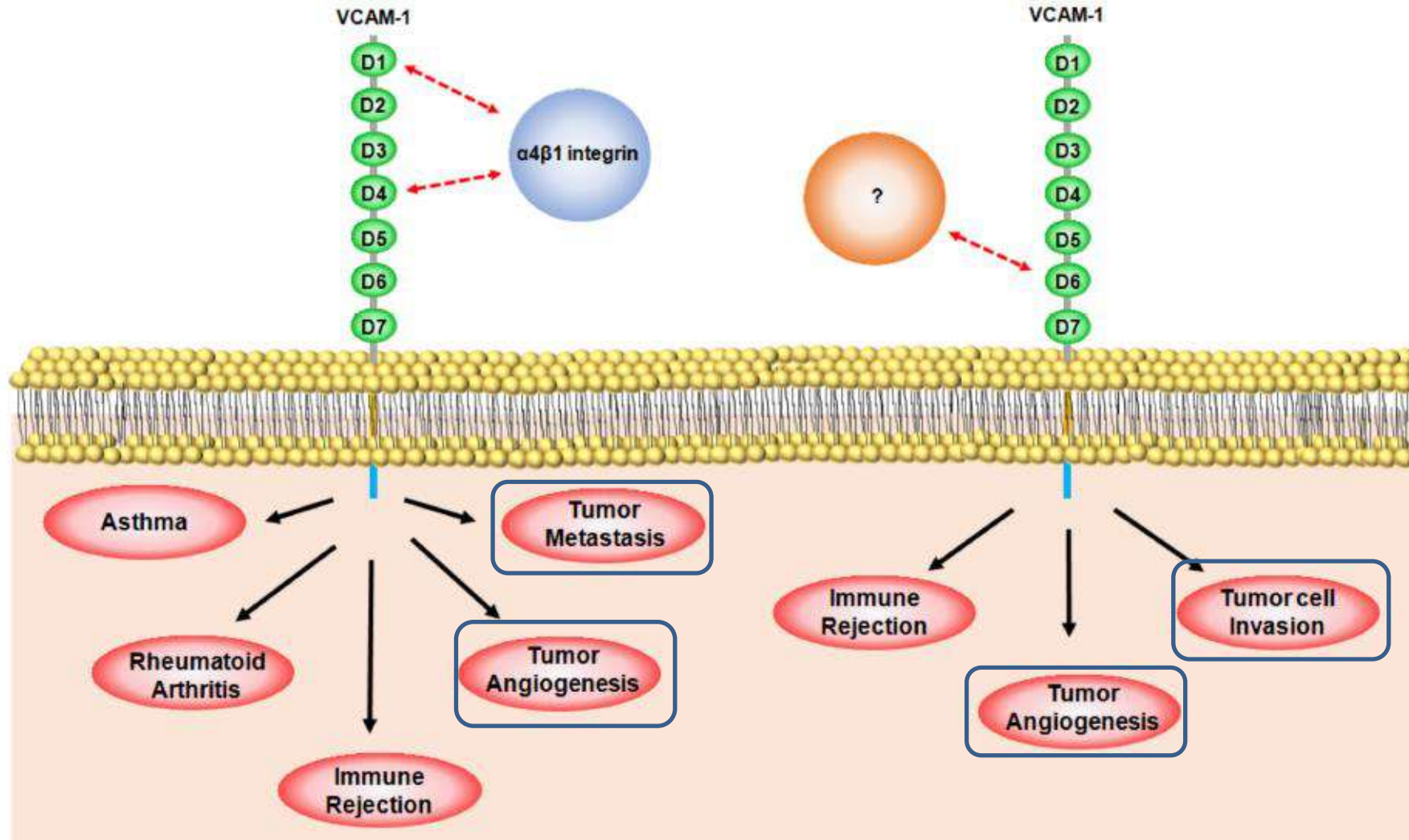
Kong et al., 2018.

**In Renal cell
carcinoma.**

Wu T 2007.

Tumor cells can escape T-cell immunity by overexpressing VCAM1, which normally mediates leukocyte extravasion to sites of tissue inflammation.

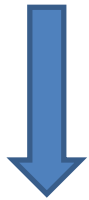
How VCAM1 works



Kong et al., 2018.

Why VCAM1 should be a target in cancer treatment?

It is highly associated with **melanomas, osteosarcoma, leukemia, and breast, renal, and gastric cancer**

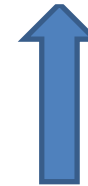


VCAM1 **promotes tumor cell adhesion** to the endothelium of blood vessels and further to facilitate cell migration

Vascular cell
adhesion molecule-1
(VCAM1)

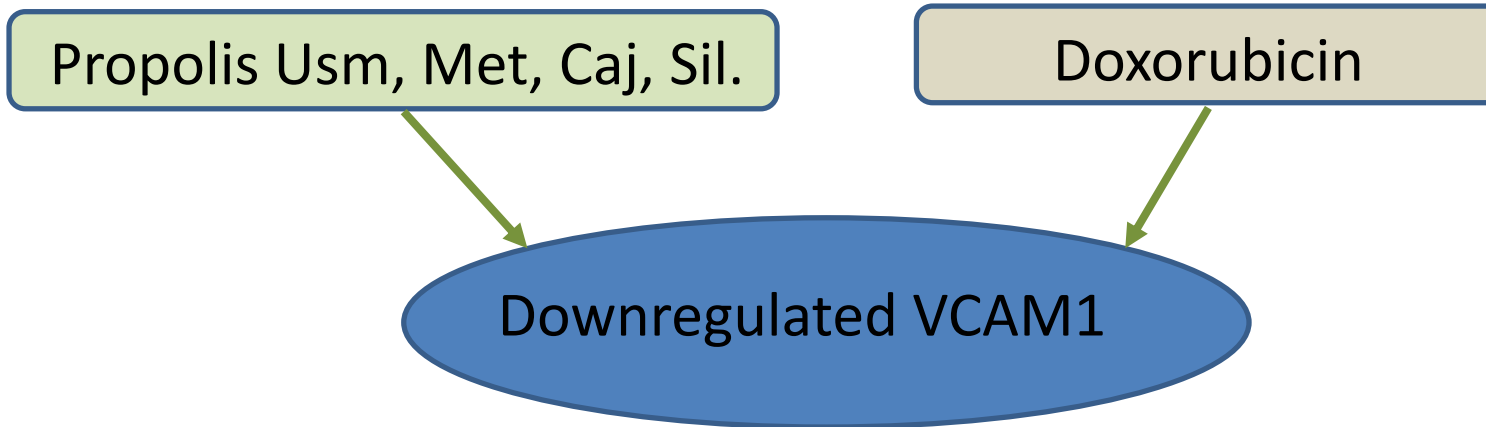


VCAM-1 is a potential target for molecular intervention in carcinogenesis.



It enhances both tumor cell growth and metastasis

Colombian propolis was found to downregulate VCAM1 expression



Pathways associated with the expression of VCAM1 may be involved in the antitumor effect of propolis samples.

Mechanism associated with chemical composition



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