



# Mechanisms underlying the cytotoxic effect of propolis on human laryngeal epidermoid carcinoma cells

Yahima Frión-Herrera, Alexis Díaz-García, Jenny Ruiz-Fuentes, Hermis Rodríguez-Sánchez & José Maurício Sforcin

To cite this article: Yahima Frión-Herrera, Alexis Díaz-García, Jenny Ruiz-Fuentes, Hermis Rodríguez-Sánchez & José Maurício Sforcin (2018) Mechanisms underlying the cytotoxic effect of propolis on human laryngeal epidermoid carcinoma cells, *Natural Product Research*, 32:17, 2085-2091, DOI: [10.1080/14786419.2017.1363749](https://doi.org/10.1080/14786419.2017.1363749)

To link to this article: <https://doi.org/10.1080/14786419.2017.1363749>



View supplementary material [↗](#)



Published online: 08 Aug 2017.



Submit your article to this journal [↗](#)



Article views: 74



View Crossmark data [↗](#)




Citing articles: 1 View citing articles [↗](#)

SHORT COMMUNICATION



## Mechanisms underlying the cytotoxic effect of propolis on human laryngeal epidermoid carcinoma cells

Yahima Frión-Herrera<sup>a</sup>, Alexis Díaz-García<sup>b</sup>, Jenny Ruiz-Fuentes<sup>c</sup>,  
Hermis Rodríguez-Sánchez<sup>c</sup> and José Mauricio Sforcin<sup>a</sup> 

<sup>a</sup>Department of Microbiology and Immunology, Biosciences Institute, UNESP, Botucatu, Brazil; <sup>b</sup>Laboratories of Biofarmaceuticals and Chemistries Productions (LABIOFAM), Havana, Cuba; <sup>c</sup>Tropical Medicine Institute Pedro Kouri, Havana, Cuba

### ABSTRACT

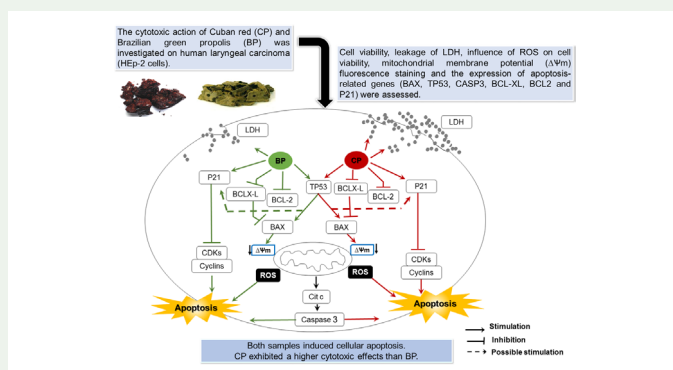
Propolis has been used as a traditional remedy for centuries because of its beneficial effects, including anticancer properties. The aim of this study was to compare the cytotoxic mechanism of Cuban red propolis (CP) and Brazilian green propolis (BP) on human laryngeal carcinoma (HEp-2) cells. Cell viability, leakage of lactate dehydrogenase, fluorescence staining, mitochondrial membrane potential ( $\Delta\Psi_m$ ) and the expression of pro/anti-apoptotic genes were assessed. Cell viability and cytotoxic assays suggested a dose-dependent effect of CP and BP extracts with a possible association of intracellular reactive oxygen species production and decreased  $\Delta\Psi_m$ . Both samples induced apoptosis via activation of *TP53*, *CASP3*, *BAX*, *P21* signalling, and downregulation of *BCL2* and *BCL-XL*. CP exerted a higher cytotoxic effect than BP extract. Our findings suggest further investigation of the main components of each propolis sample, what may lead to the development of strategies for the treatment of laryngeal cancer.

### ARTICLE HISTORY


Received 5 April 2017  
Accepted 16 July 2017

### KEYWORDS

Propolis; cytotoxicity; HEp-2 cells; apoptosis



**CONTACT** José Mauricio Sforcin  [sforcin@ibb.unesp.br](mailto:sforcin@ibb.unesp.br)

 Supplemental data for this article can be accessed at <https://doi.org/10.1080/14786419.2017.1363749>.

© 2017 Informa UK Limited, trading as Taylor & Francis Group

## 1. Introduction

Propolis has attracted a great interest in the last decades because of its several biological and pharmacological properties (Silva-Carvalho et al. 2015). Its chemical composition depends on the geographic region where it was produced and its plant source, leading to a large number of biological properties. Different types of propolis have been found, e.g. birch propolis, poplar propolis, pacific propolis, red propolis, Brazilian green propolis, Canarian propolis, and so on (Bankova 2005a). Tropical samples show unusual compositions including lignans, prenylated organic acid derivatives, terpenoids and flavonoids.

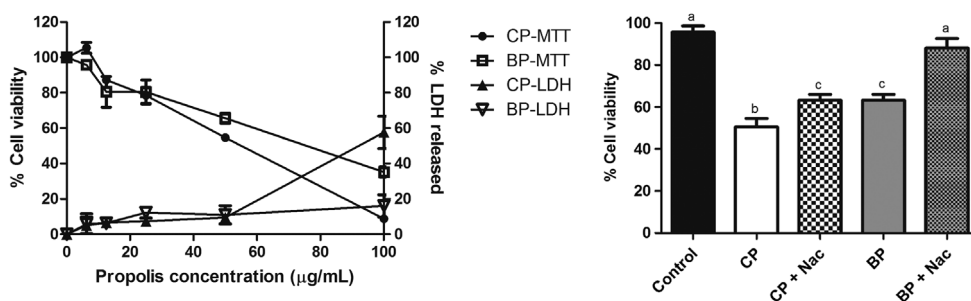
Cuba and Brazil have a huge diversity and a rich flora, and differences in the chemical composition of propolis samples in such countries have been reported (Bankova 2005b; Piccinelli et al. 2011). Cuban red propolis (CP) from the western region is derived from the floral resin of the genus *Dalbergia ecastophyllum* (Leguminosae), whose main chemical constituents are isoflavonoids (Cuesta-Rubio et al. 2007). Brazilian green propolis (BP) collected in Botucatu, south-east Brazil, is derived from *Baccharis dracunculifolia* DC. (Compositae), *Eucalyptus citriodora* Hook. (Myrtaceae) and *Araucaria angustifolia* (Bert.) O. Kuntze (Araucariaceae) (Bankova et al. 1999). The major constituents of BP are artepillin C, flavonoids, triterpenes, benzoic acid, prenyl-*p*-coumaric acid, *p*-coumaric acid, dihydrocinnamic acid, trihydroxymethoxy flavonon, tetrahydroxy flavonon (Conti et al. 2015; Sforcin 2016).

The antitumour effect of propolis has been the subject of many studies. *In vitro* studies have shown different sensitivity of tumour cells to propolis due to its chemical composition (Bankova 2005a; Frión-Herrera et al. 2015). Propolis may suppress cancer/precancerous cells proliferation (Oršolić 2010) and induce apoptosis or necrosis depending on its chemical composition. These observations led us to compare two distinct chemically characterised samples: Cuban red propolis (CP) and Brazilian green propolis (BP) on human laryngeal epidermoid carcinoma (HEp-2) cells, investigating their mechanisms of action.

## 2. Results and discussion

CP and BP exhibited a significant dose-dependent decrease in HEp-2 cells viability compared to control after 72 h, using the highest concentrations (50 and 100 µg/mL). *In vitro* LDH release from HEp-2 cells provided an accurate measure of cytotoxicity and cell membrane integrity caused by propolis samples. CP exerted a cytotoxic effect on HEp-2 cells (57.6%), while BP extract displayed a mild cytotoxic effect on these cells (16.1%) (Figure 1). The inhibitory concentrations ( $IC_{50}$ ) for CP and BP were  $56.2 \pm 7.3$  and  $64.1 \pm 4.8$  µg/mL, respectively. Our data are in agreement with previous studies showing that BP extract can reduce cell viability and increase LDH release, revealing that the cytotoxic effect on HEp-2 cells was related to membrane integrity (Frión-Herrera et al. 2015). CP cytotoxic effects may be due to its chemical composition, which contains isoflavonoids as major constituents (Cuesta-Rubio et al. 2007). Flavonoids and derivatives exert an anticarcinogenic activity; therefore, CP cytotoxic effects could be explained by the presence and proportion of isoflavonoids in our sample.

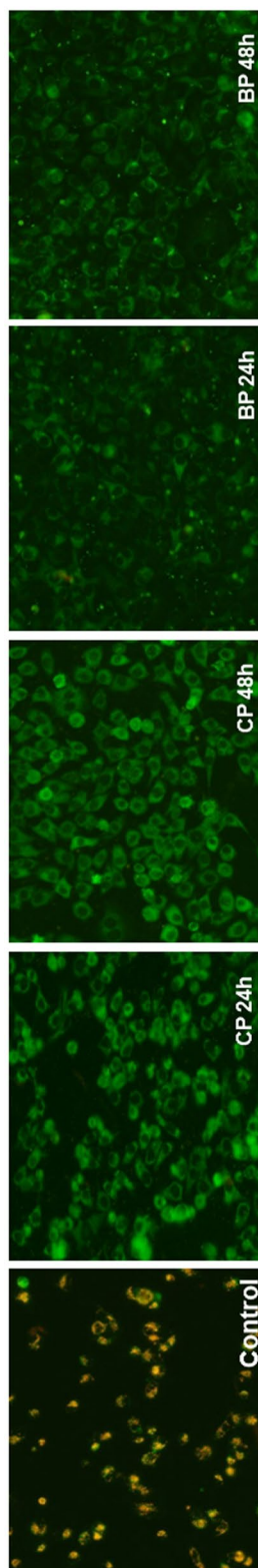
The inhibitory effect of CP and BP on cell proliferation may be related to the production of reactive oxygen species (ROS). The cytotoxic effect induced by CP and BP on HEp-2 cells after 72 h was reversed by a ROS scavenger (NAC). The co-treatment of NAC with CP and BP increased cell viability from 50.4 to 63.2% and from 63.2 to 88.1%, respectively (Figure 1).



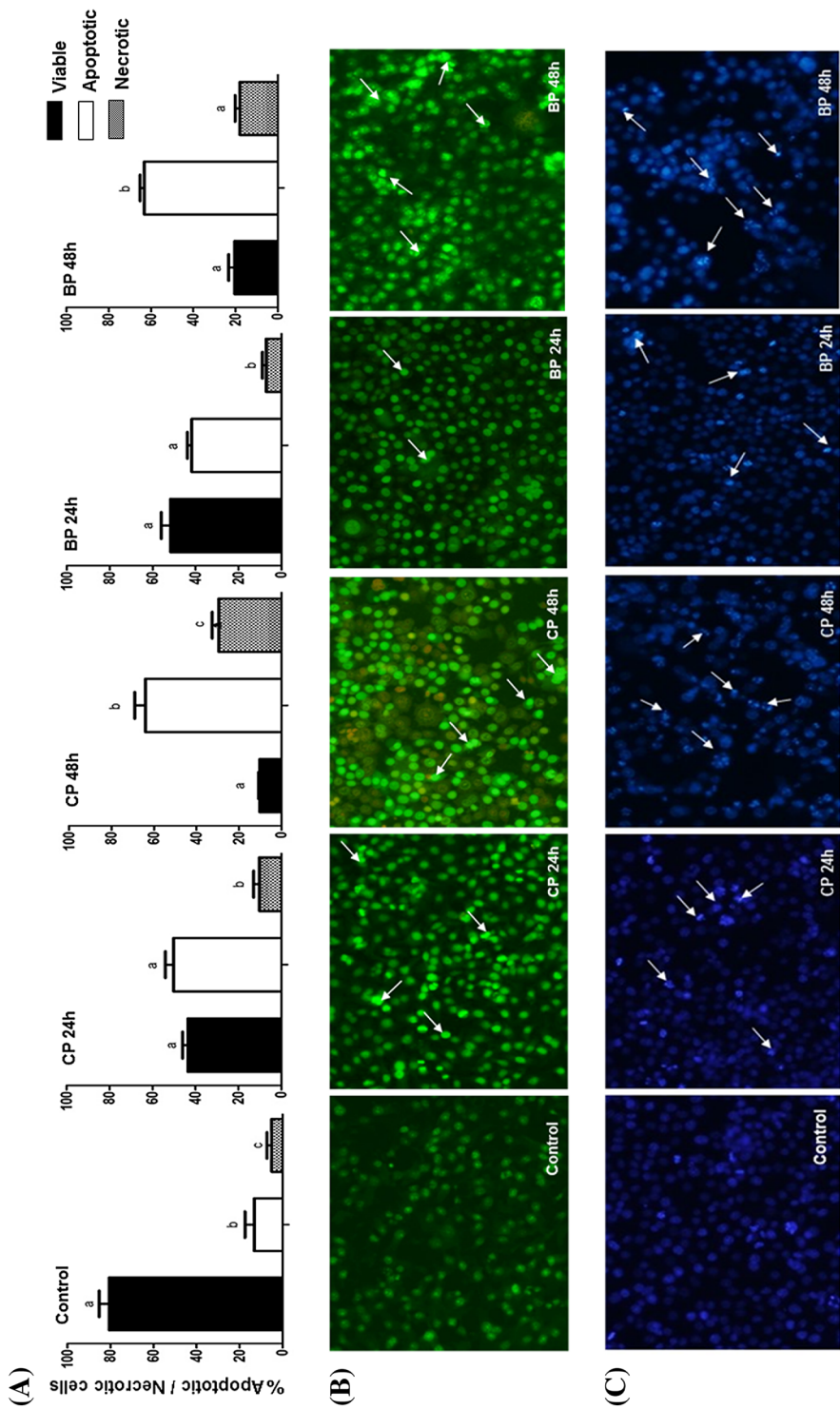
**Figure 1.** Viability and cytotoxicity (%) of HEp-2 cells after incubation with CP and BP (6.25–100  $\mu\text{g/mL}$ ) for 72 h. Cell viability was measured by MTT assay and cytotoxicity by lactate dehydrogenase (LDH) release (left). Effects of CP and BP in association with NAC on reactive oxygen species production (right). HEp-2 cells were co-incubated with CP or BP (IC<sub>50</sub>) and NAC (5 mM) for 72 h. Cell viability was measured by MTT assay and compared to NAC-treated cells. Different letters indicate significant differences between the treatments ( $p < 0.05$ ). Data are expressed as means  $\pm$  standard deviation (SD) of three independent experiments, in triplicate.

Polyphenols concentration determines the ROS-scavenging action or the occurrence of oxidative damage (Sandoval-Acuña et al. 2014). Therefore, depending on polyphenolic content and the experimental design, propolis samples could induce or inhibit ROS formation (Socha et al. 2015). Redox imbalance may lead to a loss of cell integrity and consequent cell death, and one may suggest that oxidative stress is a possible mechanism underlying the cytotoxicity induced by both CP and BP in HEp-2 cells. ROS generation can be associated with mitochondrial dysfunction and the effect of propolis samples on  $\Delta\Psi\text{m}$  of HEp-2 cells was investigated. An impairment of mitochondrial membrane integrity was observed after treatment with CP and BP extracts for 24 and 48 h, showing an increased green fluorescence that was related to mitochondrial dysfunction and cell death (Figure 2). On the other hand, a higher number of necrotic cells was found after treatment with CP after 24 h. The induction of two different types of cell death within one cell population might be a result of different expression levels or post-translational modifications of pro-necrotic and pro-apoptotic proteins in a particular cell (Su et al. 2015). Our findings corroborate the hypothesis that propolis can inhibit the growth of a variety of tumour cells via apoptosis, and it is likely that the presence of different components contribute to different pharmacological interactions, inducing mainly apoptosis with varying magnitudes (Fríon-Herrera et al. 2015).

Treatment of HEp-2 cells with CP and BP for 24 and 48 h revealed an appearance of labelled cells, representing apoptotic DNA fragmentation (Figure 3(A) and (B)). An increased number of non-viable apoptotic cells with a green fluorescence was noted after 48 h compared to 24 h (CP-24 h: 55.1%; CP-48 h: 64.0%; BP-24 h: 41.8%; BP-48 h: 63.1%). In control cultures, fewer or no apoptotic cells were observed. Additionally, the number of apoptotic cells after 24 h treated with CP (55.1%) was higher than BP-treatment (41.8%), while after 48 h an increased number of necrotic cells was observed due to CP-treatment (29.5%) compared to control and BP-treatment. DAPI staining exhibited significant alterations in HEp-2 cells (Figure 3C). Cells incubated with both samples for 48 h showed morphological changes compared to the treatment of 24 h, which included fragmentation and condensation of chromatin and nucleus, and formation of apoptotic bodies. In contrast, control cells exhibited no differences in appearance.

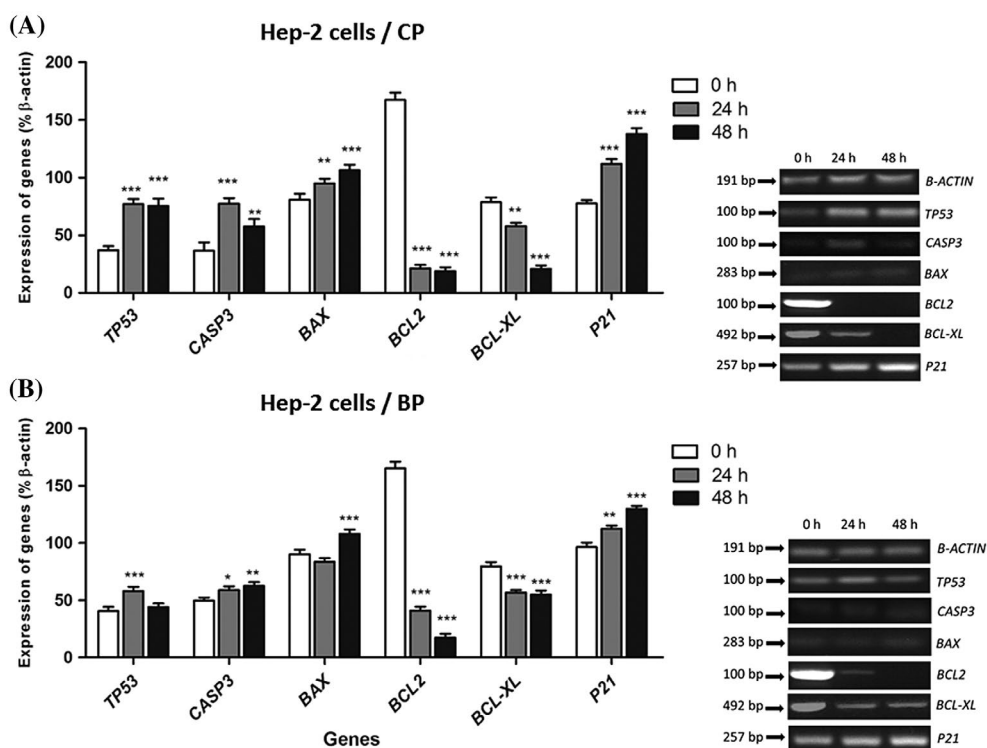


**Figure 2.** Effect of propolis CP and BP on mitochondrial membrane potential in HEP-2 cells. Cells were incubated for 24 and 48 h with  $\frac{1}{4}$  IC<sub>50</sub> of each sample. Red fluorescence represents JC-1 aggregates accumulated in mitochondria due to high  $\Delta\Psi_m$ , whereas green fluorescence represents monomeric JC-1 in the cytoplasm indicating a lowering of  $\Delta\Psi_m$ . Images are magnified at 400 $\times$ .



**Figure 3.** Morphological changes in HEP-2 cells treated with CP and BP by AO/EB and DAPI staining. Cells were incubated for 24 and 48 h with  $\frac{1}{4}$  IC50 of each propolis. A total of 200 cells were analysed and counted in triplicate, in three independent experiments. Images are magnified at 400x. (A) Number of viable, apoptotic and necrotic cells counted (%). Data are presented as mean  $\pm$  SD. Different letters represent significant differences between the groups ( $p < 0.05$ ). (B) AO/EB staining, white arrows indicate apoptotic cells. (C) DAPI staining, white arrows indicate chromatin condensation and apoptotic bodies.





**Figure 4.** Relative quantification of TP53, CASP3, BAX, BCL2, BCL-XL, and P21 mRNA levels. HEp-2 cells were treated with  $\frac{1}{4}$  IC<sub>50</sub> of CP (A) and BP (B) for 0, 24 and 48 h (left). Relative signal intensity of each gene after treatment (right). Data represent mean  $\pm$  SD ( $n = 3$ ). \*\*\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*  $p < 0.001$  vs 0 h.

Apoptosis is a physiologically active process involving activation, expression and regulation of several genes. Both CP and BP extracts drastically increased the expression of TP53, CASP3, BAX and P21. However, CP stimulated CASP3, BAX and P21 mRNA expression more efficiently than BP after 24 h. CP decreased slightly CASP3 mRNA expression after 48 h. On the other hand, CP exhibited a similar level of TP53 expression after 24 and 48 h, while TP53 expression was stimulated by BP only at 24 h. In addition, BCL2 and BCL-XL genes were downregulated by both propolis samples (Figure 4). CP induced a higher apoptosis-mediated death than BP in HEp-2 cells. These results strongly support the hypothesis that propolis effects regulating gene expression and/or modulating gene products may be related to the chemical composition of propolis samples, which is highly dependent on the flora where it was collected.

## Acknowledgements

Authors thank Prof. Christiaan Moora for assisting with the English language.

## Disclosure statement

No potential conflict of interest was reported by the authors.

## ORCID

José Maurício Sforcin  <http://orcid.org/0000-0003-3466-4895>

## References

- Bankova V. **2005a**. Recent trends and important developments in propolis research. *Evid Based Complement Alternat Med.* 2:29–32.
- Bankova V. **2005b**. Chemical diversity of propolis and the problem of standardization. *J Ethnopharmacol.* 100:114–117.
- Bankova V, Boudourova-Krasteva G, Sforcin JM, Frete X, Kujumgiev A, Maimoni-Rodella R, Popov S. **1999**. Phytochemical evidence for the plant origin of Brazilian propolis from São Paulo state. *Z Naturforsch C.* 54:401–405.
- Conti BJ, Bankova V, Sforcin JM. **2015**. Chemical composition of the same Brazilian propolis sample analyzed in 1997 and in 2012: no freezing effect. *Nat Prod Commun.* 10:1279–1280.
- Cuesta-Rubio O, Piccinelli AL, Fernandez MC, Hernandez IM, Rosado A, Rastrelli L. **2007**. Chemical characterization of Cuban propolis by HPLC-PDA, HPLC-MS, and NMR: the brown, red, and yellow Cuban varieties of propolis. *J Agric Food Chem.* 55:7502–7509.
- Fríón-Herrera Y, Díaz-García A, Ruiz-Fuentes J, Rodríguez-Sánchez H, Sforcin JM. **2015**. Brazilian green propolis induced apoptosis in human lung cancer A549 cells through mitochondrial-mediated pathway. *J Pharm Pharmacol.* 67:1448–1456.
- Oršolić N. **2010**. A review of propolis antitumour action *in vivo* and *in vitro*. *J ApiProd ApiMed Sci.* 2:1–20.
- Piccinelli AL, Lotti C, Campone L, Cuesta-Rubio O, Fernandez MC, Rastrelli L. **2011**. Cuban and Brazilian red propolis: botanical origin and comparative analysis by high-performance liquid chromatography–photodiode array detection/electrospray ionization tandem mass spectrometry. *J Agric Food Chem.* 59:6484–6491.
- Sandoval-Acuña C, Ferreira J, Speisky H. **2014**. Polyphenols and mitochondria: an update on their increasingly emerging ROS-scavenging independent actions. *Arch Biochem Biophys.* 559:75–90.
- Sforcin JM. **2016**. Biological properties and therapeutic applications of propolis. *Phytother Res.* 30:894–905.
- Silva-Carvalho R, Baltazar F, Almeida-Aguiar C. **2015**. Propolis: a complex natural product with a plethora of biological activities that can be explored for drug development. *Evid Based Complement Alternat Med.* 2015:1–29.
- Socha R, Gałkowska D, Bugaj M, Juszczak L. **2015**. Phenolic composition and antioxidant activity of propolis from various regions of Poland. *Nat Prod Res.* 29:416–422.
- Su Z, Yang Z, Xu Y, Chen Y, Yu Q. **2015**. Apoptosis, autophagy, necroptosis, and cancer metastasis. *Mol Cancer.* 14:1–14.