

## Anticancer effects of flavonoids on melanoma cells: are murine cells more sensitive compared to humans?

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### Abstract

In the past few decades, research in new drugs for treatment of oncological disorders has refocused on naturally occurring products. Among various plant-derived compounds flavonoids are considered as promising chemopreventive and chemotherapeutic agents exerting specific antiproliferative and cytotoxic activities towards various cancer cell lines. Such tumoricidal action can depend on the structure of flavonoids and their concentrations, but also on the cellular milieu and cancer type. Analysing the anticancer effects of various flavonoids on different human and murine melanoma cell lines, one further aspect influencing the antineoplastic action of flavonoids is pointed out in this article, i.e. the species origin of malignant cells. It is shown that murine melanoma cells tend to exert somewhat lower resistance to different flavonoids compared to human melanoma cells, emphasising that anticancer effects exhibited by natural polyphenolic agents in mouse cells can not be directly extrapolated to humans.

**Keywords:** Flavonoids; Melanoma; Cancer; Cytotoxicity

### Introduction

Flavonoids are polyphenolic compounds naturally present in vegetables, fruits, herbs and beverages like tea and wine, being thus regularly consumed in the common diet [1, 2]. Based on the epidemiological studies the foods rich in flavonoids contribute to reducing the risk of cancer [1-4] and various hypotheses have been proposed to explain such chemoprotective properties [5]. Due to their capability to scavenge free radicals the antioxidant action of flavonoids has been the major focus of attention [6-8]. However, emerging data indicate that these polyphenolic compounds do not act only as conventional hydrogen-donating antioxidants but may also exert modulatory actions on cells through inhibiting or stimulating signalling pathways of different protein kinases and lipid kinases, or act as prooxidants inducing mitochondria-mediated apoptosis of malignant cells [7, 8]. These activities affect cellular functions such as proliferation and differentiation, migration, angiogenesis and cell death. In this way, flavonoids may be capable of acting at different stages of carcinogenesis, by protecting DNA against oxidative damage, inactivating carcinogens, reducing tumour growth and invasion [5, 9, 10]. The precise molecular mechanisms underlying the anticancer action of flavonoids are still not thoroughly understood. It is clear that these effects may depend on the structural properties of compounds and their applied concentrations, but also on cellular milieu and incubation times. Cytotoxicity induced by flavonoids has been related both to malignant cell lines as well as the cancer

types and tumour systems [11-13]. One further important factor influencing the anticancer potency of flavonoids might be the species origin of cells. However, this aspect has not been considered within the cytotoxic analyses of flavonoids so far and therefore, the potential dependence of anticancer effects of flavonoids on the origin of cell lines (human *vs* murine) is analysed in the present paper.

### Methods

To study the possibility that anticancer action of flavonoids might be influenced by the species origin of cell lines the concentrations of various natural flavonoids causing cell growth inhibition of 50 % (IC<sub>50</sub> values) were compiled from the literature. Flavonoids have been reported to exert a huge amount of growth inhibitory effects in several human cancer cell lines; the potential cytotoxic action of these compounds on murine malignant lines has been studied to a remarkably lesser extent. Nevertheless, quantitative data for a comparative analysis were possible to extract for melanoma cells, whereas only these compounds for which the anticancer effects on both human and mouse melanoma lines have been measured were selected. Based on these inhibitory constants the mean IC<sub>50</sub> values were calculated and these data are presented in the Table.

**Table1:** Flavonoids-mediated antineoplastic activity on human and mouse melanoma cell lines

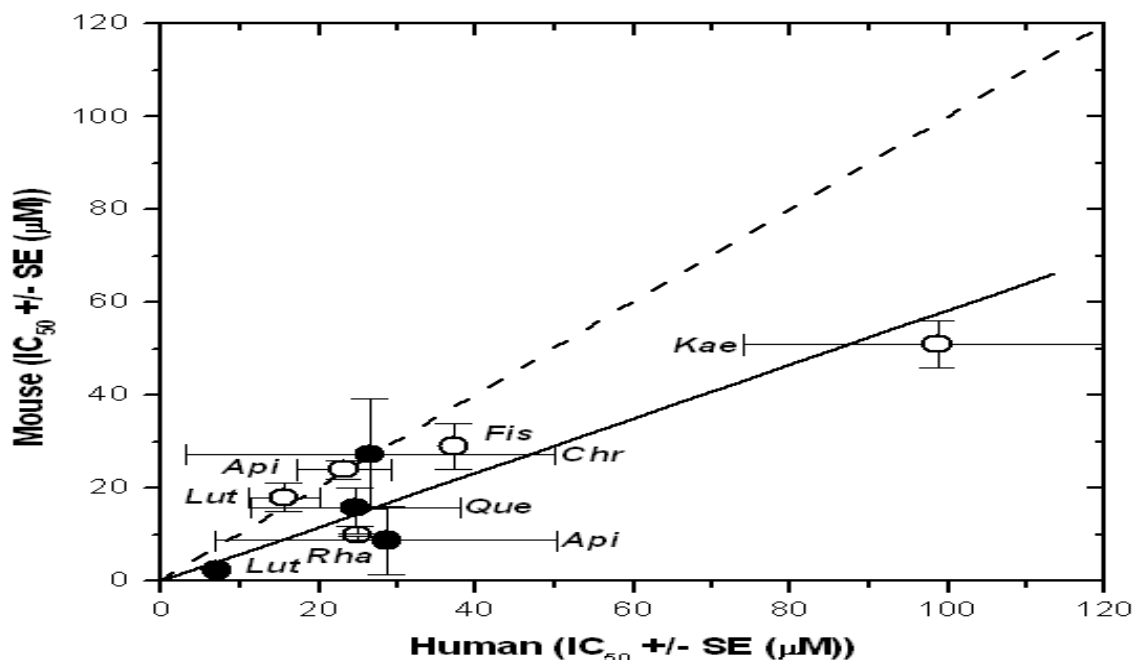
Flavonoids	Assay time	Human melanoma cells			Mouse melanoma cells			References
		Mean IC <sub>50</sub> ±SE (μM)	<i>n</i>	Cell lines	Mean IC <sub>50</sub> ±SE (μM)	<i>n</i>	Cell lines	
<i>Flavonols</i>								
Quercetin	72 h	24.68±13.32	2	SK-MEL-2, SKMEL-28	15.90±4.03	5	B16-BL6, B16 4A5, B16-F10	[7, 14, 19-22]
Fisetin	48 h	37.2	1	451Lu	29±5	1	B16	[4, 23]
Kaempferol	48 h	98.68±24.59	6	OCM-1, SK-MEL-2, A375, C32	51±5	1	B16	[1, 4, 24-26]
Myricetin	72 h	>50	3	SK-MEL-1	37.51±0.84	4	B16F10	[2, 3, 9, 10]
Rhamnetin	48 h	25	1	SK-MEL-5	10±0.3	1	B16	[4, 27]
Isorhamnetin	48 h	NE*	1	SK-MEL-2	NE	1	B16-F1	[28]
Rutin	48 h	NE	1	SK-MEL-5	NE	1	B16	[4, 27]
<i>Flavanones</i>								
Eriodictyol	72 h	>50	3	SK-MEL-1	>50	5	B16-F10, B16 4A5	[2, 3, 9, 10, 29]
Hesperetin	72 h	>50	3	SK-MEL-1	>50	4	B16-F10, B16 4A5	[2, 3, 9, 21]
Naringin	48 h	NE	1	SK-MEL-5	NE	1	B16	[4, 27]
<i>Flavones</i>								
Apigenin	48 h	23.20±6.08	4	OCM-1, C32, A375, SK-MEL-5	24.00±2.00	2	B16, B16-F10	[1, 4, 25, 27, 31]
	72 h	28.67±21.71	2	SK-MEL-2, Mel-2	8.75±7.25	2	B16-BL6, B16-F0	[19, 20, 30, 32]
Baicalein	72 h	>50	3	SK-MEL-1	30.85±2.28	4	B16-F10	[2, 3, 9, 10]
Chrysin	72 h	26.50±23.50	2	A375	27.33±11.85	3	B16-F1, B16-F0	[16, 32]
Luteolin	48 h	15.54±4.48	5	C32, OCM-1, UACC-62, A375, SK-MEL-5	18.00±3.00	2	B16, B16-F10	[1, 4, 25, 27, 31, 33]
	72 h	6.89	1	SK-MEL-2	2.3	1	B16-F10	[19, 21]
Tangeretin	72 h	>50	3	SK-MEL-1	10.72±0.29	5	B16-F10, B16 4A5	[2, 3, 5, 9, 21]

\*NE – no effect up to 100 μM

## Results and discussion

Comparative analysis of the data presented in the Table indicates that anticancer activity of flavonoids might be somewhat dependent on the species origin of tumour cell lines. This difference seems to

be not chaotic showing a clear tendency of higher sensitivity toward flavonoids for murine melanoma cells compared to human melanoma cells (see the Figure).



**Figure 1:** Comparative plot of anticancer effects of flavonoids on human and mouse melanoma cells measured at 48 hours (open circles) and 72 hours (solid circles). *Que*, quercetin; *Fis*, fisetin; *Kae*, kaempferol; *Rha*, rhamnetin; *Api*, apigenin; *Chr*, chrysin; *Lut*, luteolin

Melanoma is a tumour of great significance because it has increased alarmingly among the white population in the last 50 years [2, 3]; its incidence is rising world-wide at a rate of about 5 % per year [5]. It is the most deadly form of skin cancer: although melanoma represents less than 10 % of all skin cancers, it is responsible for more than 75 % of skin cancer-related deaths [2, 3]. Surgical intervention is the most effective treatment in its initial phases but is of little use in advanced stages of disease [2, 3], when melanoma is almost always fatal. Systemic chemotherapy is often the only viable treatment, but the lack of selective cytotoxicity often leads to intolerable side effects. Moreover, melanoma has one of the worst response rates to chemotherapy of all neoplasias because of resistance phenomena and the best response rate produced by a single-agent chemotherapy or biotherapy has been estimated to be only 16 % [2, 3, 5, 14, 15]. These problems have led to the search for new types of treatment and for new compounds with lower side effects [10, 14]. Recently, research in melanoma therapy is focused on the discovery of novel drugs able to reduce melanomas proliferative capacity and it has been shown that the progression of cancer can be arrested by the use of chemotherapeutic agents derived from herbal sources [8, 16]. Natural flavonoids are generally safe and non-toxic and have become an important part of the available arsenal of anticancer

agents [7, 17]. Indeed, among the anticancer drugs more than 50 % of the new small molecular chemical entities are of natural origin and this rate is much higher than in other areas of drug development [18].

The analysis presented in the current paper indicates that at the same doses flavonoids seem to be more sensitive to murine melanoma cells compared to human melanoma cells. This tendency may point to the differences in signalling mechanisms triggered by flavonoids in the cells of distinct species origin, but this phenomenon might also involve differences in metabolic conversion of polyphenolic compounds. It is well known that the anticancer activity of flavonoids is related not only to the flavonoids ingested but also to their metabolites [9] and this enzymatic conversion can work somewhat differently in human and mouse melanoma cells.

## Conclusions

Flavonoids are being intensively studied as potential anticancer drugs being interesting lead structures for the development of new promising candidates for treatment of cancer, including melanoma. The current paper indicates that anticancer effects of flavonoids on melanoma cells can depend on the species origin from which the

respective cell lines have been derived whereas murine cells tend to exert somewhat lower resistance than human cells. Therefore, these effects can not be extrapolated directly from mouse to humans. Moreover, the tendency presented herein can also point to some potential differences in the cellular mechanisms triggered

by flavonoids in mouse and human melanoma cells needing further clarification as a comprehensive understanding of these molecular signalling events would offer new avenues for further and more effective therapeutic intervention for this devastating disease.

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