

DOCTORAL THESIS

A mechanistic study on the anti-melanoma action of quercetin

Cao, Huihui

Date of Award:
2015

[Link to publication](#)

General rights

Copyright and intellectual property rights for the publications made accessible in HKBU Scholars are retained by the authors and/or other copyright owners. In addition to the restrictions prescribed by the Copyright Ordinance of Hong Kong, all users and readers must also observe the following terms of use:

- Users may download and print one copy of any publication from HKBU Scholars for the purpose of private study or research
- Users cannot further distribute the material or use it for any profit-making activity or commercial gain
- To share publications in HKBU Scholars with others, users are welcome to freely distribute the permanent URL assigned to the publication

**A Mechanistic Study on the Anti-melanoma
Action of Quercetin**

CAO Huihui

A thesis submitted in partial fulfilment of the requirements

for the degree of

Doctor of Philosophy

Principal Supervisor: Dr. YU Zhiling

Hong Kong Baptist University

February 2015

DECLARATION

I hereby declare that this thesis represents my own work which has been done after registration for the degree of PhD at Hong Kong Baptist University, and has not been previously included in a thesis or dissertation submitted to this or any other institution for a degree, diploma or other qualifications.

Signature: _____

Date: February 2015

ABSTRACT

The incidence and mortality rate of melanoma have increased greatly worldwide in the last thirty years. There is currently no effective treatment for malignant melanoma. Signal transducer and activator of transcription 3 (STAT3) signaling is constantly activated in human melanoma, which promotes melanoma development and progression. c-Met is a receptor tyrosine kinase (RTK), and hepatocyte growth factor (HGF) is the only known ligand of c-Met. Abnormal activation of HGF/c-Met has been implicated in melanoma metastasis. Both the STAT3 and HGF/c-Met signaling pathways are proposed as melanoma therapeutic targets. The dietary flavonoid quercetin is a bioactive compound that possesses low toxicity and exerts anti-melanoma activities. However, the anti-melanoma mechanisms of quercetin have not been fully understood. In this study, we evaluated the anti-melanoma activities of quercetin and explored the underlying molecular mechanisms.

Our results showed that quercetin treatments induced apoptosis, inhibited proliferation, migration and invasion of the melanoma cells. Mechanistic study indicated that quercetin inhibited the activation of STAT3 signaling by interfering with the phosphorylation of STAT3, thus reduced its nuclear localization. Quercetin inhibited STAT3 transcriptional activity, and down-regulated the STAT3 targeted genes such as Mcl-1, MMP-2, MMP-9 and VEGF, which are involved in cell survival, migration and invasion. More importantly, overexpression of constitutively active STAT3 partially reversed the anti-proliferative effect of quercetin, which might be correlated with the impaired effect on quercetin-mediated Mcl-1 and MMP-2 inhibition. Furthermore, quercetin suppressed A375 tumor growth and STAT3 activities in a xenografted mouse model, and inhibited murine B16F10 cells lung metastasis in mice. These findings suggest that inhibition of the STAT3 signaling pathway contributes to the anti-melanoma activities of quercetin.

Next we studied the involvement of HGF/c-Met pathway in the anti-metastasis effect of quercetin. Quercetin treatment dose-dependently suppressed HGF-induced migration and invasion of melanoma cells. Further study showed that quercetin

down-regulated the mRNA expression level of HGF and suppressed c-Met homodimerization. Quercetin also decreased c-Met protein expression, which was associated with reduced expression of fatty acid synthase. In addition, quercetin suppressed the phosphorylation of c-Met and its downstream molecules including Gab1, FAK, PAK and STAT3. Furthermore, overexpression of FAK or PAK significantly reduced the inhibitory effect of quercetin on the migration of melanoma cells. These findings suggest that suppression of HGF/c-Met signaling contributes to the anti-metastatic action of quercetin.

Besides c-Met, many other RTKs are activated in melanoma. We then further determined whether quercetin could affect the activity of other RTKs. The phospho-RTK array assay showed that quercetin treatment inhibited the activation of ROR2, Tie2, RYK, ALK, c-Ret, DDR1, DDR2, EphB4, EphA1, EphA2, EphA4 and EphA5 in A2058 cells, and EphA7, RYK, ALK and DDR1 in A375 cells. Further investigations are warranted to verify the array results, and to determine the potential roles of these RTKs in quercetin-mediated anti-melanoma properties.

Overall, our results demonstrate that quercetin exerts anti-melanoma activities. The anti-melanoma action of quercetin is, at least in part, due to the inhibition of the STAT3 and HGF/c-Met signaling pathways. Our findings provide further insights into the anti-melanoma activities of quercetin and the underlying molecular mechanisms, suggesting a potential role of quercetin in the prevention and treatment of melanoma.

ACKNOWLEDGEMENTS

First and foremost I would like to express my sincere gratitude to my principal supervisor Dr. Yu Zhiling, not only for providing me a chance to study here, but also for his continual guidance, monitor and critical advices throughout my research work.

I am indebted to my co-supervisor Dr. Anfernee Kai-Wing Tse and Dr. Anna Hiu-Yee Kwan for their generous and selfless help, valuable advices and support on my research work, also for their time and critical review on my thesis writing.

I would also like to appreciate all my colleagues in Dr. Yu's lab, especially Dr. Yu Hua, Dr. Chu Jianhong, Mr. Yuen Tszkin, Miss Su Tao, Miss Fu Xiuqiong, Ms. Chao Xiaojuan and Ms. Chen Yingjie. This big family gave me many supports and kind help throughout the period of my PhD studies.

I am particularly grateful to Dr. Chen Lei and Miss Xiao Tingting for their suggestions, supports, and encouragements. They made my studying period enjoyable and I am appreciating the friendship we developed. I also thank Ms. Sally Lee, Ms. Nickie Chan and Mr. Michael Wong, and many other SCM staff for their technical supports.

Last but not least, I would like to express my profound gratitude to my family for giving me love and supports.

TABLE OF CONTENTS

DECLARATION.....	I
ABSTRACT.....	II
ACKNOWLEDGEMENTS	IV
TABLE OF CONTENTS.....	V
LIST OF TABLES.....	IX
LIST OF FIGURES	X
LIST OF ABBREVIATIONS.....	XIV
CHAPTER 1 Introduction.....	1
1.1 Melanoma	1
1.1.1 Epidemiology of melanoma.....	2
1.1.2 Risk factors and prevention of melanoma	4
1.1.3 Current therapeutic approaches and limitations.....	6
1.2 STAT3 signaling	12
1.2.1 Structure and function of STAT3	12
1.2.2 STAT3 in melanoma progression.....	15
1.2.3 Strategies for targeting STAT3 in melanoma.....	15
1.3 HGF/c-Met signaling.....	17
1.3.1 Structure and function of c-Met.....	17
1.3.2 HGF/c-Met signaling and melanoma metastasis	21
1.3.3 Strategies for targeting c-Met in melanoma	22
1.4 Nutrition and melanoma chemoprevention.....	23
1.5 Quercetin.....	26
1.5.1 Metabolism and bioavailability	26
1.5.2 Safety	28
1.5.3 Bioactivities of quercetin	29
1.5.4 Anti-melanoma effect of quercetin.....	37
1.5.5 Inhibitory effects of quercetin on STAT3 and HGF/c-Met signalings.....	38

1.6 Hypothesis and objectives	39
CHAPTER 2 Materials and Methods	41
2.1 Materials and reagents	41
2.2 Cell culture.....	44
2.3 Cell viability assay.....	44
2.4 Apoptosis assay.....	45
2.5 <i>In vitro</i> cell migration assay—wound healing assay	45
2.6 <i>In vitro</i> cell migration assay—migration chamber assay.....	45
2.7 <i>In vitro</i> cell invasion assay.....	46
2.8 Western blot analysis	47
2.9 Preparation of cytoplasmic and nuclear fractions.....	48
2.10 Preparation of membrane protein	48
2.11 Real-time quantitative polymerase chain reaction analysis	49
2.12 Gelatin zymography.....	50
2.13 Plasmid transient transfection.....	51
2.14 Luciferase assay.....	51
2.15 Dimerization of c-Met	52
2.16 Nude mice xenografted model.....	52
2.17 <i>In vivo</i> model of lung metastasis.....	53
2.18 Phospho-RTK array analysis	54
2.19 Statistical Analysis.....	54
CHAPTER 3 Quercetin exerts anti-melanoma activities in cultured cells and in	

animal models.....	56
3.1 Abstract	56
3.2 Introduction.....	57
3.3 Results.....	60
3.3.1 Quercetin reduced cell viability in melanoma cells.....	60
3.3.2 Quercetin induced apoptosis in melanoma cells.....	62
3.3.3 Quercetin impaired the migratory and invasive capacities of melanoma cells	64
3.3.4 Quercetin exhibited anti-tumor activity in human melanoma A375 xenografted nude mouse model	68
3.3.5 Quercetin prevented murine melanoma B16F10 cell lung metastasis.....	70
3.4 Discussion and conclusion.....	72
CHAPTER 4 Inhibition of STAT3 signaling contributes to the anti-melanoma action of quercetin	74
4.1 Abstract	74
4.2 Introduction.....	75
4.3 Results.....	77
4.3.1 Quercetin reduced constitutive STAT3 phosphorylation in human melanoma cells and tumor tissues	77
4.3.2 Quercetin reduced STAT3 nuclear localization.....	89
4.3.3 Quercetin inhibited STAT3-luciferase reporter activity.....	91
4.3.4 Quercetin down-regulated the expression levels of STAT3 target genes..	93
4.3.5 Overexpression of STAT3 blunted the anti-proliferative effect of quercetin	97
4.4 Discussion and conclusion.....	102
CHAPTER 5 Involvement of the HGF/c-Met signaling pathway in the anti-metastasis effect of quercetin in melanoma	105

5.1 Abstract	105
5.2 Introduction.....	106
5.3 Results.....	108
5.3.1 Quercetin inhibited HGF-induced melanoma cell migration and invasion	108
5.3.2 Quercetin down-regulated HGF mRNA expression level, inhibited c-Met dimerization, and reduced HGF-stimulated c-Met phosphorylation	111
5.3.3 Pretreatment with quercetin reduced c-Met expression most likely through the inhibition of fatty acid synthase	117
5.3.4 Quercetin suppressed the activation of c-Met downstream molecules....	125
5.3.5 Overexpression of PAK or FAK partially reversed the inhibitory effect of quercetin on cell migration	131
5.4 Discussion and conclusion.....	133
CHAPTER 6 Phospho-RTK array study of quercetin in melanoma cells	139
6.1 Introduction.....	139
6.2 Results and discussion	143
CHAPTER 7 General discussion, Conclusion and Future plan.....	151
7.1 General discussion and Conclusion	151
7.2 Future plan	155
7.2.1 Study if other RTKs would involve in quercetin-mediated anti-melanoma properties	155
7.2.2 Study the anti-melanoma effect of the combinations of quercetin and approved drugs.....	156
REFERENCES.....	159
CURRICULUM VITAE.....	189
PUBLICATIONS	189