

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

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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: _____

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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.

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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 |