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.

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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 homo-dimerization. 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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