DOCTORAL THESIS

Baicalein Targeting TLR4 is a Potential Therapeutic Strategy for the Treatment of Obesity-associated CRC

CHEN, Minting

Date of Award:
2022

Baicalein Targeting TLR4 is a Potential Therapeutic Strategy for the Treatment of Obesity-associated CRC

ABSTRACT

Colorectal cancer (CRC) is one of the most common cancers worldwide. Obesity is a risk factor for CRC. Nearly one-third of the CRC patients is either overweight or obese. The efficacies of many current frontline treatments for CRC are reduced in the obesity-associated CRC. Currently, there is no targeted therapy for the obesity-associated CRC patients.

It is known that obesity promotes CRC development. However, the critical signaling molecule that mediates the CRC growth under obesity is not well-defined. Identifying the master regulator that controls CRC growth under obesity or HFD feeding can facilitate the development of targeted therapeutics for the cancer treatment.

In our study, lipidomics and RNA sequencing data showed that, HFD not only increases tumor weight, but also the palmitic acid level and toll like receptor 4 (TLR4) expression in the tumor tissues of CRC-bearing mouse models. The expression of TLR4 and palmitic acid level are reduced when HFD is replaced by matched control diet (CD). These concomitant changes suggest the roles of palmitic acid and TLR4 in CRC growth under HFD feeding. Subsequent studies showed that palmitic acid regulates TLR4 expression in PU.1-dependent manner. Knockdown of PU.1 abolishes the palmitic acid increased TLR4 expression. TLR4 promoter reporter assay and point mutation analysis suggest that PU.1 binding sites at -106, -172 and -204 on the TLR4 promoter are essential for the palmitic acid mediated TLR4 transcription. The role of palmitic acid/PU.1/TLR4 axis in CRC growth is further examined in cell models and animal models that are fed either HFD or palmitic acid-rich diet. Interestingly, knockout of TLR4 in the tumor tissues abolishes the HFD-increased ATP levels. Similarly, iTRAQ proteomics data showed that knockdown of TLR4 changes the metabolic enzyme profiles in the tumor tissues, which are verified by western blot analysis. Our data suggest that TLR4 mediates the growth of CRC under HFD feeding by programming the cancer metabolism. TLR4 is a potential therapeutic target for treating obesity-associated CRC.

TLR4 is a transmembrane pattern recognition receptor. TLR4 can be activated by

pathogen-associated molecular patterns such as lipopolysaccharides (LPS). Upon activation, TLR4 will be dimerized and form the activated complex (LPS.MD-2.TLR4). Indeed, LPS inserts into the hydrophobic binding pocket of the myeloid differentiation protein 2 (MD-2) that bound to TLR4. Therefore, TLR4 antagonists are designed based on the modification of the structure of lipid A4 that is the innermost regions of LPS. However, many of these lipid A analogues or the synthetic ligands have poor water solubility and bioavailability and may be toxic.

Natural small molecules that interrupt the formation of the (LPS.MD-2.TLR4) complex have been identified. However, none of them has been developed as TLR4-targeting agent. Interestingly, many of these compounds are flavonoids. Scutellaria species have high content of flavonoids. Baicalein (5, 6, 7-trihydroxyflavone) is a phenolic flavonoid found in *S. baicalensis* Georgi.

In our study, molecular docking, surface plasmon resonance biosensor analysis and bio-layer interferometry analysis demonstrated a direct physical binding between baicalein and TLR4. Cellular thermal shift assay also showed that baicalein physical binds to TLR4 and the binding of baicalein interrupts the binding of LPS to TLR4-MD-2 complex in CRC cells. Subsequent studies showed that baicalein inhibits TLR4 activity as indicated by the reduced phosphorylation of NF-κB in the CRC cells. Our data also suggest that the reduced NF-κB activity is unlikely due to the changes of the expressions of TLR4, MyD88 and TIRAP because baicalein dose not significantly affect their expression levels in CRC cells. Besides, our data showed that baicalein reduces CRC cell viability in a TLR4-dependent manner in the presence or absence of LPS. Subsequent drug–targets–disease network and protein-protein interaction network highlight hypoxia inducible factor (HIF-1α) and vascular endothelial growth factor (VEGF) are downstream of TLR4. In CRC cell models, activation of TLR4 by LPS enhances HIF-1α and VEGF expressions, which is abolished in the presence of baicalein or TLR4 inhibitor C34. In the TLR4-knockout cells, baicalein also fails to reduce the expressions of HIF-1α and VEGF. These data suggest that baicalein reduces HIF-1α and VEGF expressions in a TLR4-dependent manner. Both HIF-1α and VEGF are implicated in CRC metastasis. Indeed, wound healing assay suggests that baicalein inhibits CRC cell migration. In CRC-bearing mouse model, baicalein significantly reduces TLR4 activity in the tumor tissues and reduces the tumor size. More importantly, baicalein also reduces the metastatic markers vascular endothelial growth factor (VEGF), cluster of differentiation 31 (CD31) and matrix metalloproteinases-2 (MMP-2) in the tumor tissues. The

anti-angiogenic effect of baicalein is also demonstrated by the reduced blood vessel formation in the chick yolk sac membrane, reduced vessel sprouting in the rat aortic ring model and the cultured human vascular endothelial cells. These data strongly suggest that baicalein inhibits CRC growth and metastasis by binding to TLR4 and inhibits TLR4 activity.

Our data show that TLR4 is the master regulator that regulates the growth of CRC under HFD feeding; and baicalein inhibits TLR4 activity. Therefore, we have also examined whether baicalein reduces CRC growth under HFD feeding. The data showed that baicalein inhibits CRC growth in TLR4-dependent manner under HFD feeding.

In summary, we have demonstrated that TLR4 is a master regulator for CRC growth under HFD feeding by programming the cancer metabolism. We have also demonstrated that baicalein directly binds to TLR4, inhibits TLR4 activity, and inhibits CRC growth and metastasis *via* the HIF-1α/VEGF signaling pathway. Furthermore, we have verified that baicalein also reduces CRC growth in TLR4-dependent manner under HFD feeding. Our study provides strong scientific evidence to support the translation of baicalein into TLR4-targeting therapeutics agent for the treatment of CRC and obesity-associated CRC.

Key words: Obesity; Colorectal cancer; Toll like receptor 4; TLR4; Palmitic acid; PU.1; Metabolism; Baicalein; hypoxia-inducible factor-1α; HIF-1α; vascular endothelial growth factor; VEGF