Abstract

Colonctal cancer is the third most common cancer and the second leading cause of cancer death in the United States. Metastatic colorectal cancer (mCRC) is still a non-curative disease (5-year survival rate of 11.6%), so novel treatments are urgently needed. Dickkopf 2 (DKK2) is identified as a secreted modulator of Wnt via binding to LRP5/6. DKK2 can either stimulate or inhibit Wnt signaling depending on cell environment. In colorectal cancer, DKK2 and LRP5 are both upregulated. The interaction of DK2 and over expressed LRP5 activates Wnt signaling. Recent study shows that Wnt signaling activation contributes to the resistance toward immune checkpoint inhibitor therapy. Additionally, DK2 is observed to deactivate NK cell and CD8+ T cell in mCRC. Furthermore, another study reported that DK2K2 promote angiogenesis in mCRC through stimulating lactate secretion.

Here, we describe our efforts to develop mirror image peptide (D-peptide) inhibitors against DKK2 for mCRC. We will screen for such inhibitor using mirror-image phage display, which requires chemical synthesis of mirror-image version of the functional domain of DKK2 (C-terminus cysteine rich domains, DKK2C, 88aa). Both the L-DKK2 and D-DKK2C have been chemically synthesized by using a combination of solid-phase peptide synthesis and native chemical ligation. The Wnt reporter activity assay shows that synthetic L-DKK2 has similar activity in inhibiting Wnt signaling as recombinant DK2C does. We will now use D-DKK2C to screen for D-peptide inhibitor. Subsequently, the D-peptide inhibitor will be tested on several cell cultures to determine its efficacy on inhibiting Wnt signaling under LRP5 overexpression, activating NK cell and inhibiting angiogenesis. Compared to monoclonal antibodies, D-peptides have several advantages, including lower manufacturing cost, lower immunogenicity, and higher diffusion into solid tumors. Therefore, D-peptide drug may provide a promising alternative to monoclonal antibodies for treating mCRC.

Introduction

Metastatic Colorectal cancer (mCRC) (3-year survival rate of 11.6%)

1. Anti-PD-1 monoclonal antibody nivolumab has been FDA-approved to treat microsatellite instability-high (MSI-H) mCRC
2. Low response rate to immune checkpoint inhibitor among patients.
3. Wnt signaling pathway is one of the resistance mechanisms.
4. Severe immune related adverse events.
5. Slow pharmacokinetics of antibody drug relates to adverse events.

Anti-angiogenesis therapy

1. Anti-VEGF antibody therapies such as bevacizumab have been used in the treatment of metastatic colorectal cancer.
2. Some patients are observed to have resistance to anti-angiogenic drug targeting VEGF pathway.

Immune checkpoint inhibitor therapy

1. Inhibiting NK cell activation through interaction with LRP5.
2. Suppressing tumor immune response to anti-PD-1
3. L-DKK2.
4. Inhibits the formation of metastatic tumors

Project Goal

Screening method: mirror image phage display

1. Chemically synthesize mirror image of DKK2C (C-terminus cysteine rich domain interacting with LRP5/6)
2. Phage display with D-DKK2C to identify L-peptide binders
3. Synthesize the D-counterparts of the winning L-peptides
4. Measure affinity of D-peptides for L-DKK2C by SPR

Total Chemical Synthesis of D-DKK2C (88aa, 5 disulfide bonds)

Bioactivity Test On Synthetic L-DKK2C

Inhibition of Wnt 3a activity mediated by recombinant L-DKK2 and synthetic L-DKK2C

Future work

D-peptide inhibitor from mirror image phage display