Anti-DKK2 D-peptide Inhibitor, One Drug With Multiple Effects on Metastatic Colorectal Cancer Treatment

Weiliang Xu and Michael S. Kay

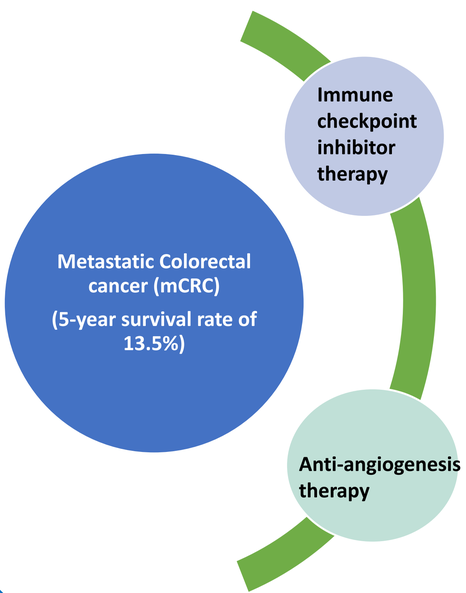
Department of Biochemistry, University of Utah, Salt Lake City, the United States.

Abstract

Colorectal 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-curable disease (5-year survival rate of 13.5%), 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 LRP6 are both upregulated. The interaction of DKK2 and over expressed LRP6 activates Wnt signaling. Recent study shows that Wnt signaling activation contributes to the resistance toward immune checkpoint inhibitor therapy. Additionally, DKk2 is observed to deactivate NK cell and CD8+ T cell in mCRC. Furthermore, another study reported that DKK2 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 domain, DKK2C, 88aa). Both the L-DKK2C 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 DKK2C 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 LRP6 overexpression, activating NK cell and inhibiting angiogenesis. Compared to monoclonal antibodies, D-peptides have several advantages, including lower manufacturing cost, lower immunogenicity, and higher diffusivity into solid tumors. Therefore, D-peptide drug may provide a promising alternative to monoclonal antibodies for treating mCRC.

Introduction



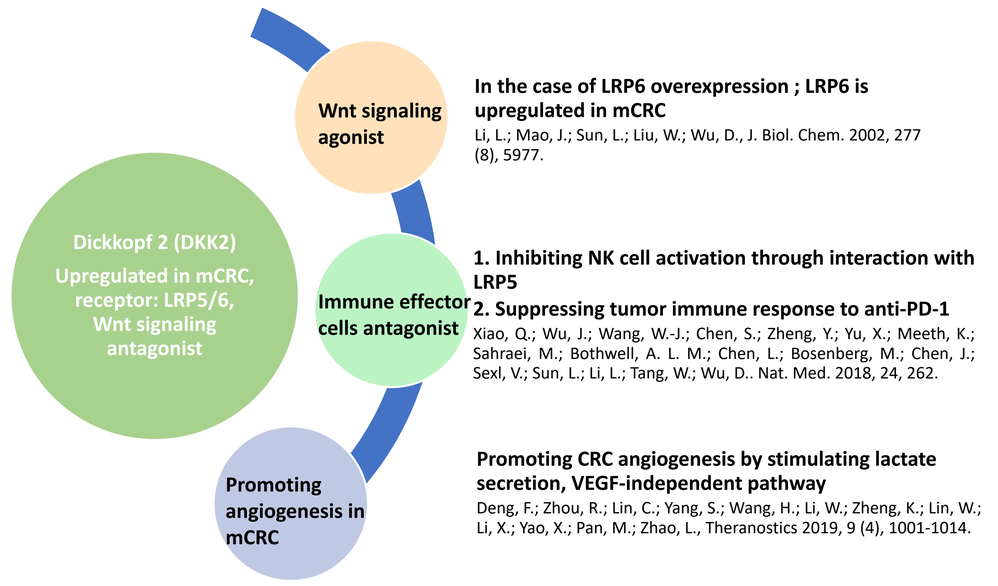
1. Anti-PD-1 monoclonal antibody nivolumab has been FDA-approved to treat microsatellite instability-high (MSI-H) mCRC

2. Low response rate to immune checkpoint inhibitor among patients.
Wnt singalling pathway is one of the resistance mechanism

3. Severe immune related adverse events
Slow pharmacokinetic of antibody drug relates to adverse events.

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

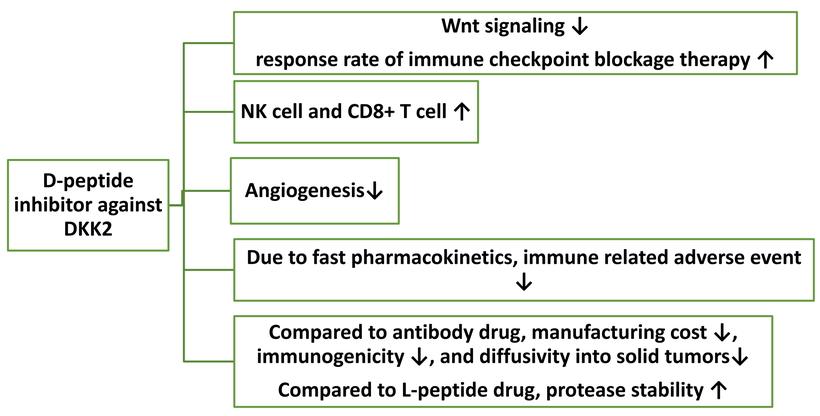


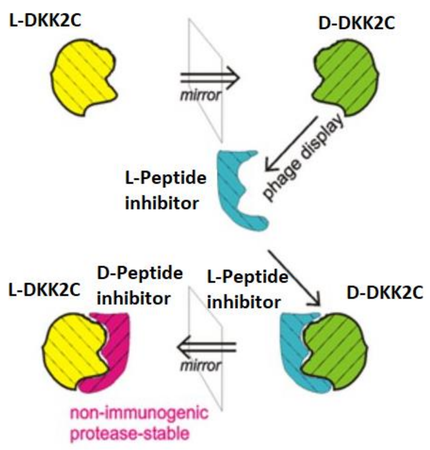
In the case of LRP6 overexpression ; LRP6 is upregulated in mCRC
Li, L.; Mao, J.; Sun, L.; Liu, W.; Wu, D., J. Biol. Chem. 2002, 277 (8), 5977.

1. Inhibiting NK cell activation through interaction with LRP5
2. Suppressing tumor immune response to anti-PD-1
Xiao, Q.; Wu, J.; Wang, W.-J.; Chen, S.; Zheng, Y.; Yu, X.; Meeth, K.; Sahraei, M.; Bothwell, A. L. M.; Chen, L.; Bosenberg, M.; Chen, J.; Sexl, V.; Sun, L.; Li, L.; Tang, W.; Wu, D.. Nat. Med. 2018, 24, 262.

Promoting CRC angiogenesis by stimulating lactate secretion, VEGF-independent pathway
Deng, F.; Zhou, R.; Lin, C.; Yang, S.; Wang, H.; Li, W.; Zheng, K.; Lin, W.; Li, X.; Yao, X.; Pan, M.; Zhao, L., Theranostics 2019, 9 (4), 1001-1014.

Project Goal



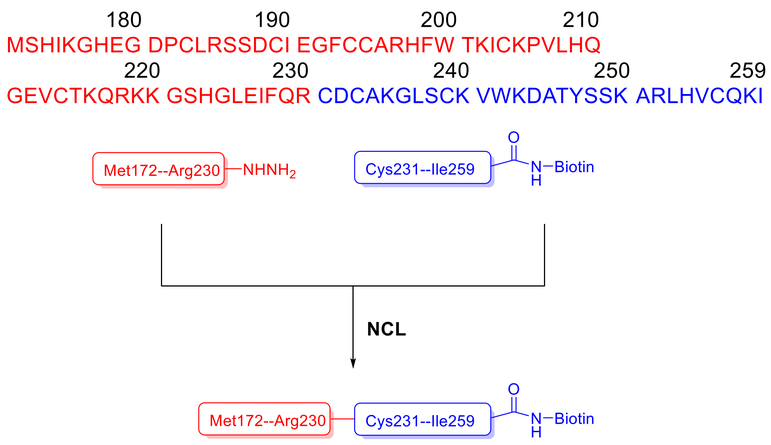


http://www.ipb.hhu.de/en/research/methods/phagedisplay-screening.html

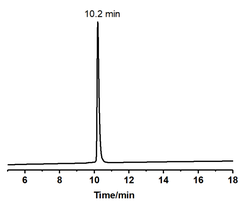
Screening method: mirror image phage display

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**

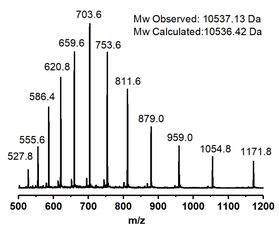
Total Chemical Synthesis of D-DKK2C (88aa, 5 disulfide bonds)



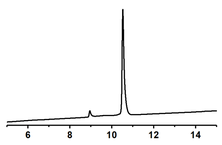
isolated yield: 40%



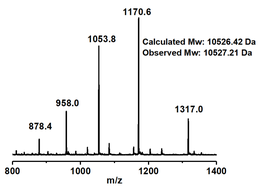
C4, 20%-70% ACN in 30min



refolding, 100mM Tris, 20mM NaCl, 500mM Arg, 1mM KCl,1mM GSSG, 9mM GSH, pH=8.0, 4°C, 24hr



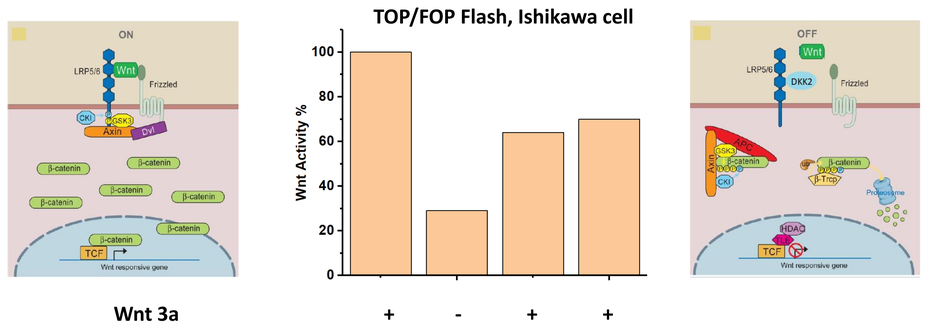
Folded protein after HPLC purification, 2mg, 20%



ESI-MS, -10Da, 5 pairs of disulfide bond formed

Bioactivity Test On Synthetic L-DKK2C

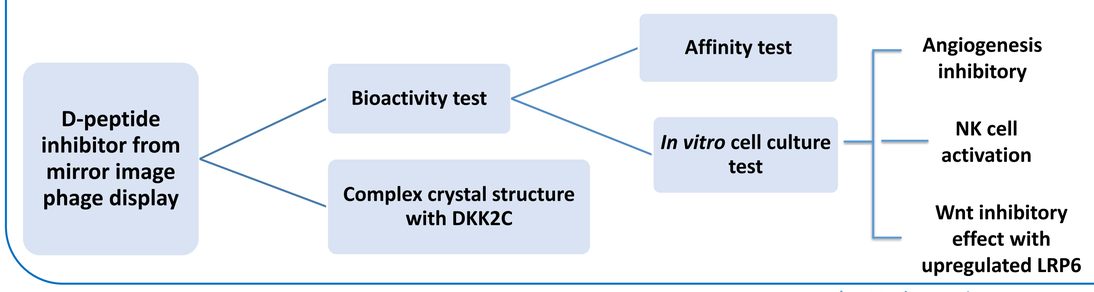
Inhibition of Wnt 3a activities mediated by recombinant L-DKK2C and synthetic L-DKK2C



Wnt 3a	+	-	+	+
Recombinant DKK2 50nM	-	-	+	-
Synthetic L-DKK2 50nM	-	-	-	+

Wnt signaling figures are adapted from: MacDonald, B. T.; Tamai, K.; He, X., Dev. Cell 2009, 17 (1), 9-26.

Future work



Scan this code to connect with Weiliang (Timo) on LinkedIn