Curcumin analogues for targeting cancer metastasis via G-alpha protein signaling

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Introduction

Heterotrimeric G proteins act as molecular switches that modulate numerous cellular signaling pathways [1]. G-protein signaling is initiated and mediated by the binding of guanine nucleotide Exchange Factors (GEFs) to inactive G-proteins which accelerates the rate of exchange of GDP for GTP [1,2]. Gαi proteins have been demonstrated to enhance Akt activation [3], remodel the actin cytoskeleton, and mediate cell migration [4,5], making them a desirable pharmacological target for inhibiting cancer metastasis. A GDP-selective Gαi binding peptide, KB-752, has previously been demonstrated to enhance spontaneous nucleotide exchange of Gαi subunits [6]. Several specific contacts between a conserved TWXE/DFL and Gαi1 have been shown to be critical for nucleotide exchange [6]. An intramolecular hydrogen bonding network within the α-helical TWXE/DFL motif involving threonine 4 (T4) and aspartate 7 (D7) serve to orient both tryptophan (W5) and phenylalanine (F8) toward the Gα binding face of the peptide, burying W5 within a hydrophobic pocket formed by F215, L249, and I253 of Gαi1, while F8 likewise resides within a hydrophobic environment established by W211, I212, and F215 of Gαi1. A library of peptidomimetic small molecules utilizing a core structure from the natural product curcumin was constructed. Computer-assisted drug discovery focused the library to identify curcumin analogues that bind Gai1 in a fashion similar to the tryptophanyl moiety of KB-752. The analogues are being synthesized and prepared for analysis.

References: [1] Oldham WM, Hamm HE. Nat Rev Mol Cell Biol. 2008, 9(1):60-71. [2] Tall GG, Krumins AM, Gilman AG J Biol Chem. 2003, 278:8356–8362. [3] Anai M, et al. J Biol Chem. 2005, 280:18525–18535. [4] Enomoto A, et al. Dev Cell 2005, 9:389–402. [5] Jiang P, et al. Cancer Res. 2008 68:1310–1318. [6] Garcia-Marcos M, Ghosh P, Farquhar M. PNAS. 2009, 106 (9) 3178-3183

Acknowledgements

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Results

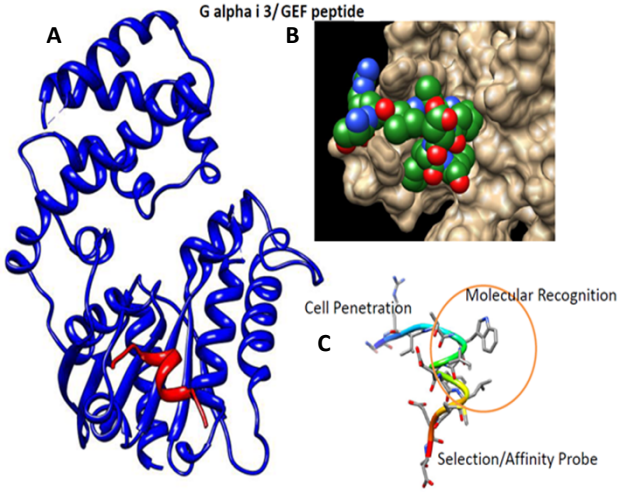
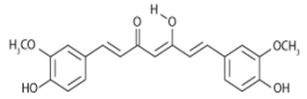


Figure 1 A) Binding of Gαi3 to GEF peptide. B) Binding interface. C) Functional motifs of GEF peptide used for small molecule design.



Curcumin

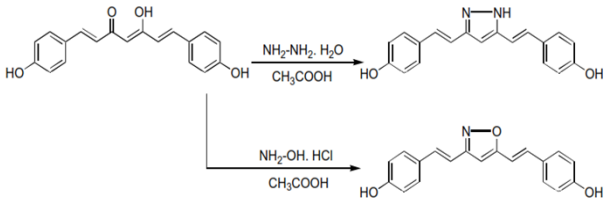


Figure 2 Curcumin and synthesis of analogues.

Discussion

•Generated computational models of G-α proteins and a small library of GEF-like peptides.

• Identified two regions spanning residues 201-215 and 248-259 in Gαi confer specificity to GEF-like peptides.

•A conserved TWXE/DFL motif is required for utilizing stacking interactions with W211 and F215 on Gαi3 and the residues that flank the TWXE/DFL motif confers binding specificity.

•The binding interface of GEF served as the basis for the core structure of small molecules.

We believe that small molecules targeting GEF/Gα interactions provide a novel and alternative strategy to improve therapeutic efficiency of anti-cancer agents by localizing premetastatic and metastatic cancer cells.

Future Directions

Future experiments will seek to determine whether curcumin analogues demonstrate inhibitory action on G-protein signaling and cancer metastasis.

Fluorimetric assays will be used to assess inhibition of G-protein signaling.

To assess actions on metastatic activity a MCF-7 cell-based wound heal assay will be used .

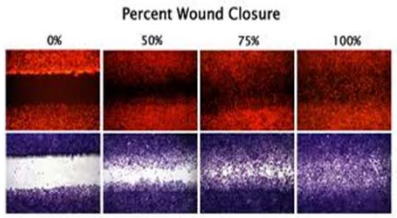


Figure 3 Experimental strategy. For wound heal assay