CARNA BIOSCIENCES

News Release

January 25, 2019
Carna Biosciences, Inc.

Carna Announces Publication in Scientific Reports Describing the Development of Novel Biosensors for Cell-Based Assay System

Carna Biosciences announced that research results discovered at CarnaBio C-Lab, a research center in the U.S. established to develop novel drug discovery technologies, have been published in *Scientific Reports*, a scientific journal of the Nature Publishing Group, on January 24. The paper describes the development of a novel screening system for BRAF inhibitors with safer profiles utilizing our split luciferase complementation technique. The article, titled "Development of Highly Sensitive Biosensors of RAF Dimerization in Cells", is available online at www.nature.com/articles/s41598-018-37213-2.

"We are excited by the extraordinary scientific achievements of the C-Lab team. These findings suggest huge potential of the split luciferase complementation technique in the field of drug discovery research," said Dr. Kohichiro Yoshino, President and CEO of Carna Biosciences.

Masaaki Sawa, Ph.D., Chief Scientific Officer of Carna Biosciences and co-author of the journal article, noted, "Our study provides comprehensive data on inhibitor-induced RAF dimerization and improves our current understanding of the mechanism of action of RAF inhibitors. Moreover, our novel assay system will be a useful tool to develop next-generation RAF inhibitors that promote no or minimal dimerization, and hence exhibit improved safety and durable efficacy.

Carna Biosciences is a biopharmaceutical company focusing on the discovery and development of small molecule drugs to address serious unmet medical needs in oncology and autoimmune diseases.

Abstract:

RAF protein kinases are key components of the RAS-RAF-MEK-ERK signaling cascade, which transmits signals from cell-surface receptors to promote cell proliferation and survival. Among three isoforms of RAF [ARAF, BRAF, and CRAF (also known as RAF1)], BRAF has attracted the greatest attention as a therapeutic target, since BRAF mutation have been identified in 8% of human tumors, particularly in ~60% of melanomas. ATP-competitive BRAF inhibitors dabrafenib and vemurafenib induce remarkable clinical responses in patients with BRAF-mutated melanoma and received US Food and Drug Administration (FDA) approval for the treatment of this disease. However, they often produce side effects, such as cutaneous squamous cell carcinoma, and moreover, continuous administration leads to drug resistance. Previous studies revealed RAF dimerization induced by inhibitors is the mechanism underlying these undesirable events. Thus, preventing RAF dimerization is an effective strategy for developing safer and more durable next-generation RAF inhibitors, and a screening system for RAF dimer detection is needed. The published paper describes a highly sensitive and quantitative bioassay for detecting RAF dimers utilizing enhanced click beetle luciferase complementation. C-Lab team developed novel biosensors to detect all possible combinations of BRAF, BRAF(V600E), and CRAF, which enabled the quantitative comparison of levels of dimerization between RAF isoforms or

compounds. Using this novel assay system, the team successfully profiled commercially available RAF inhibitors, together with other experiments, yielded the following insights:

- RAF dimerization is promoted by nine of ten commercially available RAF inhibitors, with various intensities and temporal patterns, but the drugs share a common preference for CRAF-containing dimers.
- Inhibitor-induced RAF dimerization does not always correlate with RAF enzyme inhibition.
- CRAF, but not BRAF, makes a major contribution to the inhibitor-induced paradoxical activation of downstream MEK-ERK signaling.

This simple and highly-sensitive assay system will be a powerful tool for drug screens to develop RAF inhibitors with minimal or no effects on dimerization, paradoxical activation, and resistance.

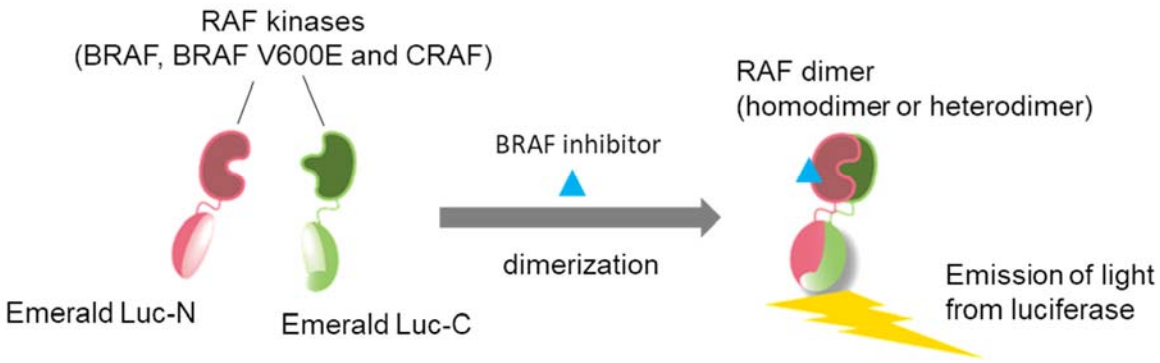

Figure. Highly Sensitive Biosensors of RAF dimerization

Publication

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