Inhibiting the Receptor Tyrosine Kinase-like Orphan Receptor 2 (ROR2) enzyme with small molecules for the treatment of cancer

Brett Rodgers[1] , Michiru Nishita[2], William H. Bisson[1]

[1]Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR 97331, USA.
[2] Physiology and Cell Biology, School of Medicine, Kobe University, Kobe 650-0017, Japan.

Abstract: Based on recent findings, **our hypothesis is that the inhibition of the Receptor tyrosine kinase-like orphan receptor 2 (ROR2) enzyme can lead to tumor suppression.** ROR2 are trans-membrane proteins that are part of the receptor tyrosine kinase (RTK) family. ROR2 is generally not expressed in most normal adult tissues and represents a new target in the WNT signaling pathway. In order to test our hypothesis, we have developed a model using transiently transfected HEK-293T cells to screen for inhibition of ROR2-mediated downstream signaling. The inhibition of ROR2 activation, through canonical WNT pathway, will be studied by monitoring the level of activated-ROR2-induced phosphorylation of G-protein coupled-receptor Kinase 2 (GRK2) compared to untreated cells. The research proposed is significant because it will lead to novel lead compounds for multiple tumor types.

Introduction

- Studies indicate that in cancers driven by canonical Wnt signaling, ROR2 expression is increased.
- The elevated expression of ROR2 is correlated with tumor progression in multiple tumor types.
- Decreased ROR2 expression (siRNA) in melanoma suppresses cancer in mice.

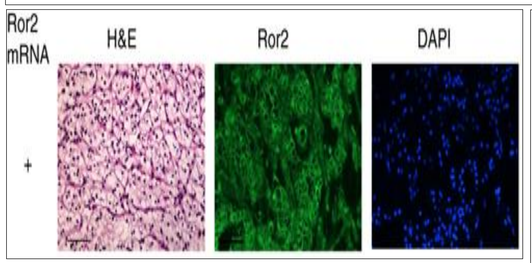

Figure 1: ROR2 expression in renal cell carcinoma (RCC) tissue. ROR2 is a transmembrane surface protein expressed (Wright et al. Oncogene 2009).

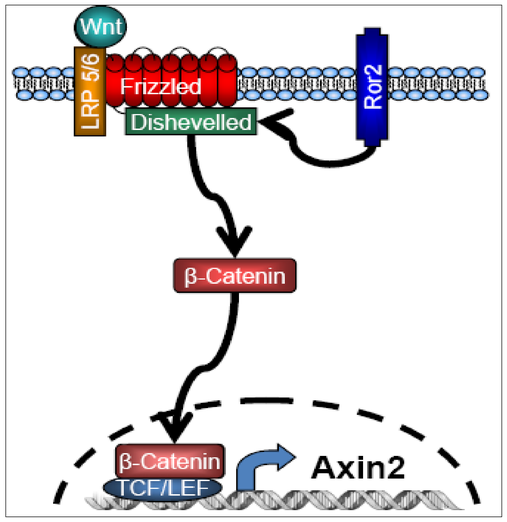

Figure 2: Model of ROR2/Wnt3A canonical signaling in RCC cells. ROR2 expression in RCC cells results in increased beta-Catenin stabilization. This event will favor tumor progression (Rasmussen et al. JBC 2013).

Goals

- We will develop a cellular assay to screen small molecule compounds for ROR2 inhibition.
- We will monitor phosphorylation of downstream protein, G protein coupled receptor kinase 2 to study ROR2 activation.
- We will test the role of ROR2 expression and activation with and in the absence of WNT3A ligand (canonical signaling pathway).

Assay Development

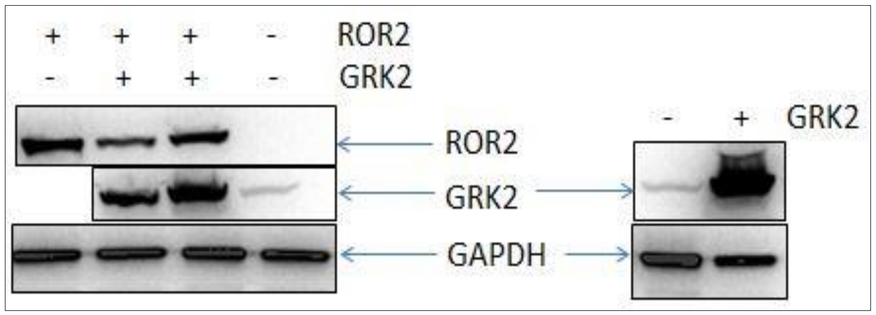

Figure 3: Expression of ROR2 and GRK2 in HEK293T cells. ROR2 and GRK2 were transiently co-transfected (1:1). Minor levels of GRK2 expression in HEK293T cells. GAPDH was used as expression control.

Virtual Ligand Screening (VLS)

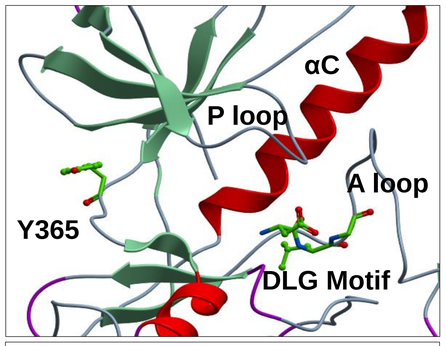

Figure 4: Homology modeling of ROR2 kinase domain (KD) in the active conformation. Homology modeling was based on human TRKB-KD template (ICM 3.7).

After VLS, a series of type I inhibitor candidates were selected for *in vitro* testing.

High Throughput Screening (HTS)

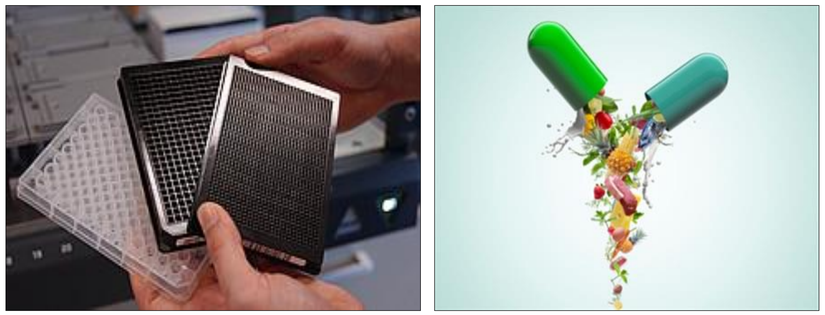

Figure 5: A Kinase Focused Library and Natural Compound Library. Specific small molecule compound libraries will be screened to find potential ROR2 inhibitor candidates.

	tissue
MDA-MB-453	**breast**
HCT116	**colon**
786-0	**kidney**

Table 1: **ROR2+ human cancer cell lines.** Example of cell lines to be tested for suppression of growth.

Conclusions

- An assay based on HEK293T cells was developed to detect ROR2-induced phosphorylation of downstream protein GRK2.
- Individual type I inhibitors and specific small molecule compounds libraries were selected for *in vitro* screenings.
- The best candidates will be tested for suppression of growth in a selected panel of ROR2+ cancer cell lines.