AKT inhibition reduces cell motility in CD90+ Hepatocellular Carcinoma

Jonathan T Lifferth[1] †, Naotoshi Nakamura[2] †, Darren R Tyson[1], Naohiko Koshikawa[3], Takashi Suzuki[2], Carlos F Lopez[1], Vito Quaranta[1]

***Short Abstract* — Subtypes of Hepatocellular carcinoma (HCC) can be classified by expression of stem cell surface markers EpCAM+, CD90+, or lack thereof. Mathematical modeling based on time-series RRPA data suggests that metastasis in CD90+ HCC cell lines is dependent on AKT activity. Statistical analysis of HCC cell speed demonstrates that treatment with MK2206, an AKT inhibitor, results in reduced cell speed in CD90+ HCC cells and not in EpCAM+ cells.**

***Keywords* — AKT; CD90; EpCAM; EphA2, hepatocellular carcinoma, cancer biology, systems biology, reverse phase protein array.**

I. Background

Hepatocellular Carcinoma (HCC) is typically characterized by elevated expression of EGFR and EphA2. Additionally, HCC cell lines can be classified into three subtypes based on the presence of cancer stem cell markers: EpCAM+ (epithelial, tumorigenic), CD90+ (mesenchymal, metastatic), and EpCAM-/CD90- (neutral) [1, 2].

II. Abstract

Using PySB [3] and PyDREAM [4], we constructed a mathematical model of EGFR and EphA2 signaling pathways in HCC. Model parameters were based on time-series reverse phase protein assay (RPPA) data measuring concentrations of molecules in the EGFR/EphA2 signaling pathways including phosphorylated and unphosphorylated forms of EGFR, EphA2, AKT, and others. This model showed asymmetrical signal dependence on AKT in CD90+ cell lines. This indicates that metastasis (measured as cell speed) of HCC cell lines is more dependent on AKT activity in CD90+ cells than in EpCAM+ cells. To evaluate the effect of an AKT inhibitor (MK2206) on cell speeds between the two subtypes, bright field microscopy images were captured of two HCC cell lines (1 EpCAM+ and 1 CD90+) at 3 concentrations (0 uM, 1 uM, and 2.5 uM). Additionally, to compare cell speeds without treatment, bright field microscopy images were captured for six HCC cell lines (4 EpCAM+ lines and 2 CD90+ lines) over 48.5 hours at 30 minute intervals. Images were segmented using iLastik [5]. Cell coordinate identification and cell tracking were performed with TrackMate, an ImageJ plugin [6].

Kernel density estimates (KDE) of the distribution of mean cell speeds for all cell lines found that certain cell lines experience significant variability between experiments. This variability was quantified by calculating and comparing Kolmogorov-Smirnov values for mean cell speed distributions between each experiment for each cell line. KDEs were also used to compare the JHH6 (CD90+) and JHH7 (EpCAM+) cell lines under varying concentrations of MK2206. These KDEs demonstrate a shift in cell speed distribution towards lower speeds in the JHH6 cell line with increasing concentration of MK2206. No clear trend is observed for JHH7. Linear regression between cell mean speeds identified a reduction of cell speed in the JHH6 cell line (P=0.01) not in the JHH7 cell line (P=0.16). Mean cell speed in JHH6 cells decreased by 10.3% at 1μM and 17.7% at 2.5μM.

III. Conclusion

Here we demonstrate that AKT inhibition with MK2206 reduces cell motility in CD90+ HCC cells and not in EpCAM+ HCC cells. This finding reveals that detection of CD90 or EpCAM in a specific HCC tumor may be useful in improving efficacy of targeted treatment of HCC. The use of MK2206 or other AKT inhibitors merits further investigation for treating CD90+ HCC. Additionally, researchers must consider how experimental sensitivity of certain cell lines (JHH4 and Huh7) may result in significant variation between cell motility behavior in HCC.

References

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[1]Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN.
[2]Center for Mathematical Modeling and Data Science, Osaka University, Osaka 580-8531, Japan.
[3]Department of Life Science and Technology, Tokyo Institute of Technology
† These authors contributed equally to this work.