Activating Ras Mutations *in vitro* and in Cancer

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***Short Abstract* — Two classes of Ras mutations increase Ras activation and have *in vitro* transformation potential: GAP insensitive and fast-cycling Ras. While GAP insensitive mutants are commonly found in tumors, fast-cycling mutants are rarely found. It is unclear why fast-cycling mutants are not more prevalent in cancer. Our quantitative model of the oncogenic Ras signaling module predicts differential Ras activation in a manner dependent upon expression level and mutant class. Quantitative, single-cell experiments confirm the predicted patterns of Ras pathway activation. These results suggest that spontaneous fast-cycling mutations fail to cause cancer as they induce a comparably small increase in Ras activation.**

***Keywords* — Ras, cancer, single cell measurements**

I. BACKGROUND

RAS point mutations are found in approximately one third of all tumors [1]. Such mutations cause increased Ras activation (i.e., an increased fraction of total Ras bound to GTP) and subsequent activation of downstream pathways, such as the ERK/MAPK pathway [2]. These mutations and their effects are believed to play a causal role in the development of cancer [3-4].

Despite thirty years of extensive experimental investigation [2], there remain many fundamental, unexplained questions in Ras biology. For example, *in vitro* transformation assays suggest that two classes of Ras point mutants should have cancerous potential: GAP insensitive mutants and fast-cycling mutants [5]. Although GAP insensitive mutants are found in one third of all tumors [1], fast-cycling Ras mutants are rarely observed [6].

II. SUMMARY OF RESULTS

Our model uses ODEs to describe the Ras signaling module, has been validated against experimental data, and has made novel predictions that have been experimentally verified [7]. Here, we use it to study Ras activation due to a GAP insensitive or fast-cycling Ras mutant.

A. Analysis of in vitro transformation assay

For an *in vitro* transformation assay, mutant Ras is exogenously expressed in a cell line that natively expresses wild-type Ras. Approximating these levels of wild-type and mutant Ras in our model, the model predicts that both GAP insensitive and fast-cycling Ras cause high levels of Ras activation (for example, approximately 60% and 55% of total Ras bound to GTP for GAP insensitive and fast-cycling Ras, respectively).

B. Analysis of spontaneous Ras mutation

Approximating wild-type and mutant Ras expression levels consistent with a spontaneous mutation at one *ras* locus, our model predicts that GAP insensitive Ras mutants cause much more Ras activation than a fast-cycling Ras mutation (for example, 20% and 5% of total Ras bound to GTP for GAP insensitive and fast-cycling Ras, respectively).

C. Experimental assessment

To test these predictions, we used flow cytometry to make quantitative, single-cell measurements of Ras pathway activation (assessed by pERK) as a function of upstream Ras mutant (either fast-cycling or GAP insensitive). As predicted, the GAP insensitive mutant caused a strong increase in Ras activation at even the lowest levels of expression. Also as predicted, the fast-cycling mutant caused a minimal increase in Ras activation at the lowest levels of expression, but cells with high expression levels had pERK levels comparable to GAP insensitive Ras.

III. CONCLUSION

The model predicted that the intensity of a Ras signal differs between *in vitro* and *in vivo* contexts. Quantitative, single cell measurements observe the predicted patterns of Ras pathway activation. These novel observations suggest that a spontaneous fast-cycling Ras mutant would produce considerably less Ras activation than a spontaneous GAP insensitive mutant and could explain why only one of two classes of Ras mutant with *in vitro* transformation potential is actually found in tumors.

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